Study on Sorcin Mediated Pathway of Multidrug Resistance in Gastric Carcinoma

Thesis submitted

by

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Doctor of Philosophy (Science)

Department of Life Science and Biotechnology

Faculty Council of Science

Jadavpur University

Kolkata, India

2025

DEDICATED TO

This thesis is lovingly dedicated to the cherished memory of my dearest late father, **Late Dilip Kumar Ghosh.** His firm belief in my potential, endless encouragement, and enduring values have been my constant source of strength. It was his dream to be by my side throughout this journey and witness my achievements. Though he is no longer here, I hope he is proud of me as his presence remains my guiding light, inspiring me every step of the way.

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CERTIFICATE FROM THE SUPERVISOR

This is to certify that the thesis entitled **"Study on Sorcin Mediated Pathway of Multidrug Resistance in Gastric Carcinoma"** submitted by **Ms Sushmita Ghosh**, who got her name registered on 26th November, 2021 **(Index No. 116/21/LifeSc./27)** for the award of Ph.D. (Science) degree of Jadavpur University is absolutely based upon her own work under the supervision **Dr. Vilas D Nasare** and that neither her thesis nor any part of the thesis has been submitted for any degree/ diploma or any other academic award anywhere before.

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ACKNOWLEDGEMENT

First and foremost, I thank God for his blessings, strength, and guidance throughout my doctoral journey. I am deeply grateful to all the wonderful people who have supported and encouraged me along the way. Their guidance, kindness, and help have not only made this thesis possible but have also shaped me in ways that will stay with me forever.

I am deeply honoured and grateful to my venerable supervisor, **Dr. Vilas D. Nasare**, Senior Scientific Officer in the Department of Pathology and Cancer Screening at Chittaranjan National Cancer Institute, Kolkata. His constant guidance, unwavering support, and encouragement have been the driving force behind the successful completion of my thesis. **His** deep knowledge and expertise in Cancer Biology have significantly shaped my research, and his innovative ideas, constructive feedback, patience, and motivation have made this journey truly enriching. His generosity in sharing his time and resources, including essential funding, has been instrumental in the successful completion of my thesis. I am also sincerely thankful to my research advisory committee for their insightful suggestions and encouragement, which have helped refine my work and strengthen my understanding throughout this journey.

I extend my heartfelt thanks to the Director of Chittaranjan National Cancer Institute (CNCI), **Dr. Jayanta Chakrabarti**, for allowing me to carry on my work at such an esteemed organization.

I am deeply appreciative of my Co-principal Investigators and clinical collaborators, **Dr. Jayanta Chakrabarti, Dr. Neyaz Alam, Dr. Partha Nath** and **Dr. Kalyan Kusum Mukherjee**, from the Department of Surgical Oncology and Medical Oncology, Chittaranjan National Cancer Institute, Kolkata, for their assistance in designing clinical study, collecting tissue samples and epidemiological data from participating families. Special thanks to **Dr. Raya Banerjee** for her assistance in sample procurement and subsequent histopathological analysis, from the Department of pathology, Chittaranjan National Cancer Institute, Kolkata. I am grateful to the clinicians, faculty members, and support staff of the hospital and library for their invaluable

support in providing essential resources for my research. I also extend my heartfelt gratitude to the patients who generously donated their tissue samples, making our research possible.

I extend my regards to the eminent scientists **Dr. Rathindranath Baral, Dr. Nabendu Murmu, Dr. Dona Sinha, Dr. Sutapa Mukherjee, Dr. Prosenjit Saha, Dr. Arpita Chandra and Dr. Ugir Hossain SK** for their scientific criticism, experimental support, and guidance. Many thanks to the staffs of Central Research Instrument Facility (CRIF), CNCI.

I am deeply grateful to CNCI for awarding me a five-year fellowship under the **Institute Fellowship programme**, which supported me as a Junior and Senior Research Fellow during my Ph.D. This fellowship provided vital financial assistance, including a stipend, funds for consumables, travel, and contingencies, enabling me to successfully pursue my research endeavours.

I extend my heartfelt gratitude to **Dr. Parthiba Basu, Dr. Salil Raha, Dr. Arkadeep Mitra, and Dr. Dipak Som** from the University of Calcutta; **Dr. R. Suresh Kumar** and **Dr. Suresh Hedau** from the National Institute of Cancer Prevention and Research, ICMR, Noida, Delhi; and **Dr. Sunil Kumar** from ICAR-Indian Agricultural Statistics Research Institute, New Delhi. Their invaluable guidance, timely advice, and constant encouragement have been pivotal throughout my academic journey. Their unwavering support, insightful feedback, and profound expertise have significantly contributed to the successful completion of my degree. I am deeply appreciative of their mentorship, patience, and steadfast willingness to provide clarity and direction whenever it was most needed.

I would like to express my sincere gratitude to **Dr. Sutapa Mahata**, Guest faculty, Department of Biotechnology at Sidho Kanho Birsa University Purulia, for her unwavering support and valuable guidance to this study. I deeply appreciate the invaluable guidance and camaraderie of all the members of my laboratory, especially I am grateful to **Dr. Arpana Sharma, Dr. Sinjini Sarkar, Dr. Pranab Kumar Sahoo, Ms. Ranita Pal, Ms. Tanuma Mistry, Ms. Trisha Chowdhury, Ms. Madhurima Ghosh and** staffs **Mrs. Sangita Bhaduri** for their unwavering encouragement and assistance throughout my Ph.D. journey.

I extend my heartfelt gratitude to my dear friends: **Mr. Atal Behari Sinha**, **Mr. Samudra Pal**, **Ms. Rina Das**, **Ms. Rita Biswas, Mr. Shantilal Das,** and **Mr. Bikas Kabi**. Your stimulating discussions, unwavering moral support, friendship and constant motivation have been invaluable throughout my doctoral journey. The enjoyable moments we shared, even amidst our busy and strenuous schedules, provided profound comfort and rejuvenation, enabling me to sustain momentum and navigate this journey with renewed energy and enthusiasm.

Finally, I wish to express my heartfelt gratitude to my family, whose unwavering support has been my anchor throughout this journey. To my parents, **Sri Dilip Kumar Ghosh** and **Smt. Usha Ghosh**, your blessings, guidance, and constant inspiration have been the cornerstone of my strength. To my beloved siblings, **Ms. Neha Ghosh**, **Ms. Riya Ghosh**, and **Mr. Partho Ghosh**, your boundless love, encouragement, and belief in me have been a source of endless motivation. Each of you has been a shining light in my life, and I am forever grateful for your presence and unconditional support. I am truly fortunate to have such wonderful family by my side.

Sushmita Ghosh

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PREFACE

This thesis marks the culmination of my doctoral research on the role of Sorcin as a multidrug resistance (MDR) indicator in gastric carcinoma, conducted at the Department of Pathology and Cancer Screening, Chittaranjan National Cancer Institute, Kolkata, India. The journey toward completing this research has been both challenging and rewarding, deeply shaping my understanding of cancer biology and multidrug resistance mechanisms. My motivation to pursue this area of study stemmed from my fascination with the complexities of cancer treatment resistance and my desire to contribute to the development of more effective therapeutic strategies.

Gastric cancer (GC) remains a significant global health challenge, ranking as the fifth most commonly diagnosed cancer worldwide and a leading cause of cancer-related mortality. While its incidence is gradually declining, India remains one of the contributors, ranking fourth globally, with a disproportionately high mortality rate. Despite advancements in systemic chemotherapy, the failure of treatments over time, mainly due to the development of multidrug resistance (MDR), continues to impede effective management. MDR considerably diminishes the efficacy of conventional chemotherapeutic agents such as 5-fluorouracil, cisplatin, and paclitaxel, contributing to poor patient outcomes.

The mechanisms underlying MDR are complex, involving increased efflux, changes in drug metabolism, genetic mutations, and the presence of resistant cancer stem cells. Additionally, polymorphisms in MDR-related genes, such as ABCB1, have been linked to poor clinical outcomes in GC patients undergoing chemotherapy. While ATP-binding cassette (ABC) transporters like P-glycoprotein (P-gP) and multidrug resistance-associated proteins (MRPs) are critical mediators of MDR, their inhibition has not fully reversed resistance, suggesting the involvement of additional molecular players.

Among these, Sorcin, a calcium-binding protein, has emerged as a potential biomarker and therapeutic target in MDR cancers, including GC.

Overexpression of Sorcin has been shown to modulate MDR transporters and the ERK signaling pathway, promoting cancer cell survival and drug resistance. However, the precise mechanisms by which Sorcin contributes to MDR remain poorly understood. Given its pivotal role in tumor progression, metastasis, and chemoresistance, further exploration into Sorcin's function in GC could provide valuable insights for overcoming MDR and improving treatment outcomes.

The research presented in this thesis aims to elucidate the role of Sorcin as a biomarker for identifying MDR in GC patients and to explore its mechanism of action in modulating drug resistance via the ERK signalling pathway. By investigating the clinicopathological features, expression levels of Sorcin, P-gP, and ERK1/2, and the molecular interactions between these factors in drug-resistant GC cell lines, this study seeks to expand our understanding of Sorcin's potential as a therapeutic target in GC. Additionally, identifying Sorcin as a biomarker may help detect the MDR phenotype in GC patients more effectively.

In conclusion, I hope that this research serves as a meaningful contribution to the ongoing exploration of cancer resistance mechanisms and supports future therapeutic advancements.

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LIST OF FUGURES

LIST OF ABBREVIATIONS

5-FU	:	5-Fluorouracil	CSC	:	Cancer Stem Cells
ABCB1	:	ATP Binding Cassette Subfamily B Member 1	CTSZ	:	Cathepsin Z
ACD	:	Accidental Cell Death	DMEM	:	Dulbecco's Modified Eagle Medium
ACT	:	Adjuvant Chemotherapy	DMSO	:	Dimethyl Sulfoxide
AGCR	:	Anatomical Gene Expression Resource	ECOG	:	Eastern Cooperative Oncology Group
AGS	:	Human Gastric Adenocarcinoma Cells	EDTA	:	Ethylenediaminetetraacetic Acid
AGS/5FUR	:	5-Fluorouracil Resistant AGS cells	EGFR	:	Epidermal Growth Factor Receptor
AGS/DoceR	:	Docetaxel Resistant AGS cells	ELISA	:	Enzyme-Linked Immunosorbent Assay
AGS/oxaR	:	Oxaliplatin Resistant AGS cells	EMT	:	Epithelial-Mesenchymal Transition
AJCC	:	American Joint Committee on Cancer	ER	:	Endoplasmic Reticulum
Akt	:	Protein Kinase B	ERK	:	Extracellular Signal-Regulated Kinase
ALDH1	:	Aldehyde Dehydrogenase 1	F-12K	:	Ham's F-12K Nutrient Mixture
ANXN	:	Annexins	FBS	:	Fetal Bovine Serum
ATF6	:	Activating Transcription Factor 6	FDA	:	Food and Drug Administration
AURKA/B	:	Aurora Kinase A/B	FGFR	:	Fibroblast Growth Factor Receptor
Bax	:	Bcl-2-Associated X Protein	Fli	:	Flightless
Bcl-2	:	B-cell lymphoma 2	FLOT	:	5-FU-leucovorin/Oxaliplatin/Docetaxel

Bip	:	Binding immunoglobulin Protein	FOXO	:	Forkhead Box O
BSA	:	Bovine Serum Albumin	GAPDH	:	Glyceraldehyde 3-phosphate dehydrogenase
CACNB1	:	Calcium Voltage-Gated Channel Auxiliary Subunit Beta1	GC	:	Gastric Cancer
CaMKII	:	Calcium/Calmodulin-Dependent Protein Kinase II	GRB2	:	Growth Factor Receptor-Bound Protein 2
CECT	:	Contrast-enhanced computed tomography	GRP78	:	Glucose-regulated Protein 78
Co-IP	:	Co-immunoprecipitation	H&E	:	Hematoxylin and Eosin
CREB1	:	cAMP Response Element-Binding Protein 1	H. pylori	:	Helicobacter pylori
CRs	:	Complete Responders	HEK293	:	Human Embryonic Kidney 293 Cells
HER2	:	Human Epidermal Growth Factor Receptor 2	NFAT	:	Nuclear Factor of Activated T Cells
HR	:	Hazard Ration/Hazard Risk	NF-κB	:	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
IAP	:	Inhibitor of Apoptosis Proteins	NR	:	Non- Responders
IC50	:	Half-Maximal Inhibitory Concentration	NSCLC	:	Non-Small Cell Lung Cancer
IDT	:	Integrated DNA Technologies	NTD	:	N-Terminal Domain
IHC	:	Immunohistochemistry	OD	:	Optical Density
IP3R	:	Inositol 1,4,5-Trisphosphate Receptors	ORR	:	Objective Response Rate
KRAS	:	Kirsten Rat Sarcoma Viral Oncogene Homolog	OS	:	Overall Survival
LAGC	:	Locally Advanced Gastric Cancer	PARP	:	Poly (ADP-ribose) Polymerase

LVI	:	Lymphovascular Invasion	PBS	:	Phosphate-Buffered Saline
MAPK	:	Mitogen-Activated Protein Kinase	PEF	:	Penta-EF hand family
MDR	:	Multidrug Resistance	PFS	:	Progression Free Survival
MDR1	:	Multidrug Resistance protein 1	P-gP	:	P-glycoprotein
MEK	:	Mitogen-Activated Protein Kinase Kinase	PI3K	:	Phosphatidylinositol 3-Kinase
MET	:	Mesenchymal-Epithelial Transition Factor	PIC	:	Protein Inhibitor Cocktail
miRNA	:	microRNA	PKA	:	Protein kinase A
MMP2/9	:	Matrix Metalloproteinases 2 and 9	PLK1	:	Polo-Like Kinase 1
MRI	:	Magnetic Resonance Imaging	PMCA	:	Plasma Membrane Calcium ATPases
MSI	:	Microsatellite Instability	PMSF	:	Phenylmethylsulfonyl Fluoride
MTT	:	(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) Assay	PNI	:	Perineural Invasion
NACT	:	Neoadjuvant Chemotherapy	PRs	:	Partial Responders
NaF	:	Sodium Fluoride	PSRC1	:	Proline and Serine-Rich Coiled-Coil Protein 1
NBD	:	Nucleotide Binding Domains	pTNM	:	Pathologic Tumor-Node-Metastasis
NCCN	:	National Comprehensive Cancer Network	qRT-PCR	:	Qualitative and Quantitative Polymerase chain reaction
ncRNA	:	Non-coding RNA	RAF	:	Rapidly Accelerated Fibrosarcoma
RECIST	:	Response Evaluation Criteria in Solid Tumors	RCD	:	Regulated Cell Death
Rh123	:	Rhodamine 123	TRAP1	:	Tumor Necrosis Factor Receptor-Associated Protein 1
RI	:	Resistance Index	TMB	:	Tumor Mutation Burden

RIPA	:	Radioimmunoprecipitation Assay	TMD	:	Transmembrane Domain
RyR	:	Ryanodine Receptor	TME	:	Tumor Microenvironment
SCBD	:	Sorcin Calcium Binding Domain	TBST	:	Tris-Buffered Saline with Tween 20
SD	:	Standard Deviation	TCGA	:	The Cancer Genome Atlas
SDS-PAGE	:	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis	TKIs	:	Tyrosine kinase inhibitors
SE	:	Standard Error	US	:	Upfront Surgery
SERCA	:	Sarco/Endoplasmic Reticulum Calcium ATPases	VEGF	:	Vascular Endothelial Growth Factor
SH2	:	Src Homology 2	WB	:	Western Blot
SI	:	Serosal Invasion	WHO	:	World Health Organization
siRNA	:	Small Interfering RNA	ZEB1	:	Zinc Finger E-box-Binding Homeobox 1
Sorcin	:	Soluble Resistance-related Calcium-binding Protein	α-SMA	:	Alpha-Smooth Muscle Actin
SOS	:	Son of Sevenless			
SOX2	:	SRY-box transcription factor 2			
SRCC	:	Signet Ring cell Carcinoma			
STAT3	:	Signal Transducer and Activator of Transcription 3			
SYL1	:	Synaptotagmin-Like Protein 1			
TA	:	Tubular Adenocarcinoma			

CHAPTER 1
INTRODUCTION

Gastric Cancer (GC) is a one of the leading causes of cancer related deaths worldwide and most prevalent disease in Asia. According to GLOBOCAN 2022, it reportedly ranks 6th in age-standardized incidence rate and 7th in mortality rate worldwide. In India, among 55,047 incident cases, 36,389 were males and 18,658 were females, while among 49,276 mortality cases, 32,811 males and 16,465 females were succumbed to death (Bray et al., 2022; Grad et al., 2023). Geographically, the incidence of GC varies significantly, mostly in developing countries and this disparity is attributed to risk-factors like *H. pylori* infection, dietary habits, environmental, and genetic characteristics. Smoking and alcohol intake are additional risk factors that increase the risk of GC (Iwu et al., 2023). Based on clinicopathological characteristics, Lauren classification have identified GC into intestinal and diffuse histological subtypes which has proven to be essential for better diagnosis and treatment decision (Monster et al., 2022; Shah et al., 2020). There are no significant diagnostic approaches available for GC. However, some current classic diagnostic options are endoscopy, biopsy, imaging tests (e.g., computerized tomography and magnetic resonance imaging), and laboratory tests with blood and serum (Shin et al., 2023). Besides, survival rate for GC remains low due to late diagnosis of this disease at advanced stages mostly due to its asymptomatic nature, which makes early detection impossible to carry. Advancement has been made in the treatment approaches for advanced GC, importantly surgical resection with D2 lymphadenectomy, chemotherapy, targeted therapy, and chemoradiotherapy (Beyer et al., 2023). In cases where, chemotherapy is the foremost armamentarium for advanced GC. Adjuvant chemotherapy significantly reduces the mortality and recurrence of GC patients while improving overall survival and disease-free survival. However, perioperative neoadjuvant chemotherapy, have shown to be more effective in reducing the tumor size and stage, and improving the progression-free and overall survival, hence considered for first-line therapy over adjuvant chemotherapy. Particularly,

neoadjuvant FLOT therapy has shown higher response rates compared to other chemotherapy regimens used for GC treatment. Evidently, FLOT therapy can improve overall survival and quality of life of patients (ESOPEC et al., 2024; Mohring et al., 2023). However, neoadjuvant chemotherapy has shown variable response while facing limitations like, variable efficacy and reduced 5-year overall survival worldwide. This is likely due to heterogenous tumor histopathology and acquired multidrug resistance (MDR) phenotype in GC (Koerner et al., 2023; Lavacchi et al., 2023).

Cancer cells adopted different resistance properties as a survival strategy against chemotherapeutic drugs. Fascinatingly, it decreases the drug uptake, lowers the affinity of drug-target binding, alters the structure of the receptors, increase drug efflux, escape apoptosis; activate intracellular detoxifying system and DNA repair mechanism (Catalano et al., 2022). It has been observed that drug resistance can occur by the activation of both intrinsic (pre-existing) or acquired (induced by drugs) mechanisms. Intrinsic resistance decreases the initial efficacy of drug treatment, while acquired resistance depends on prior exposure to chemotherapy. Nonetheless, it is complicated to distinguish between intrinsic and extrinsic MDR during the course of chemotherapy. Growing evidences suggested that the combination therapy is more effective than monotherapy where the latter is vulnerable to the mechanistic properties of MDR. The mechanisms of action of different chemotherapeutics—such as alkylating agents, antimetabolites, topoisomerase inhibitors, and mitotic spindle inhibitors—are significantly impaired in drug resistant cancers, which contributes to over 90% of resistance-related mortality (Emran et al., 2022; Bukowski et al., 2020). Among many, drug efflux property was widely studied mechanism in cancer. Particularly, about P-glycoprotein (P-gP), an ABC transmembrane protein. P-gP or MDR1 encoded by ABCB1 gene is a ATP-dependent efflux pump predominantly linked to the development of resistance to anthracyclines, vinca alkaloids, actinomycin D, 5-Fluouracil, and paclitaxel. P-gP has the capacity to interact with over 20 substrates or modulators (e.g. verapamil and cyclosporin A). It generally regulates drug resistance through many signaling pathways. Presumably, proteins like ERK modulate the

transcriptional expression of P-gP (Ruan et al., 2020). To counteract drug resistance, P-gP targeted inhibitors were developed based on their specificity, affinity, and toxicity. However, these inhibitors failed in clinical trials due to issues such as toxicity and lack of efficacy (Karthika et al., 2022; Zhang et al., 2021). This failure has pushed researchers to identify more potent and effective targets for combating drug resistance in cancer therapy.

Soluble resistance-related calcium-binding protein (Sorcin) which regulates calcium homeostasis, is highly expressed in 25 cancers, particularly in multidrug-resistant cancer cells (Yu et al., 2018). It is identified to co-amplified with P-gP, contributing to drug resistance mechanisms (Zhou et al., 2019). Recent studies have shown that Sorcin independently plays a significant role in MDR, functioning as an oncoprotein (Li et al., 2023). Apparently, the most frequent genetic alteration in Sorcin is amplification which correlates with clinical features, stemness, tumor mutation burden (TMB), microsatellite instability (MSI), tumor immune microenvironment and closely associate with poor survival outcomes in cancers. Mechanistically, it has shown to directly bind several oncoproteins involve in cancer progression and drug resistance such as P-gP, STAT3, ANXN among others (Ling et al., 2021; Mao et al., 2020). In addition, it appears that Sorcin bind chemotherapeutic drugs like Doxorubicin, 5FU, vincristine, taxens reducing their effectiveness (Saravana et al., 2024; Battista et al., 2020). However, the underpinning mechanism of action of Sorcin is still dubious.

Furthermore, the activation of ERK signaling upregulate the expressions of ABC transporters, enhancing drug efflux activity while reducing the intracellular concentration of chemotherapeutic agents. Reportedly, Sorcin can elevate the activation of the ERK signaling, leading to increased cell proliferation, and drug resistance property in presence of chemotherapeutic drugs (Shabnam et al., 2028). Additionally, Sorcin mediated regulation of ERK signaling shown to promote metastasis, further contributing to MDR. This allows cancer cells to escape the primary tumor site and colonize distant organs (Tito et al., 2023; Tong et al., 2015). However, the precise mechanisms by which

Sorcin modulates ERK signalling is still under question. Moreover, it is known that: Sorcin can bind to and regulate various calcium-dependent signalling molecules, which in turn work through ERK signaling. This hypothesised that interaction between Sorcin and the ERK axis may involve in direct binding to regulate MDR mechanism in GC (Jain et al., 2023; Cheng et al., 2023; Zhang et al., 2021).

Therefore, understanding the involvement of Sorcin in GC, its role in drug resistance, probably through the ERK signalling could open new avenue to address MDR in GC. Additionally, Sorcin can be a potential biomarker to identify MDR phenotype in GC patients.

CHAPTER 2
REVIEW OF LITERATURE

2.1. Epidemiology and prevalence of gastric carcinoma

According to GLOBOCAN v1.1 2022, gastric cancer (GC) is the fifth most common diagnosed cancer and the fourth leading cause of cancer-related mortality worldwide (Ferley et al., 2024). Geographically, epidemiology of GC varies widely, with highest prevalence in East Asia followed by Eastern Europe and South America (Sekiguchi et al., 2022). In India, prevalence of GC accounts for about 5.8 per 100,000, with age-adjusted rate (AAR) of 3.0–13.2 per 100,000, which is lower than the global AAR of 4.1–95.5 (Banik et al., 2024). The estimated five-year survival rate is lower than 20% worldwide (Ilic et al., 2022).

2.1.1. Risk factors and symptoms

H. pylori infection, highly salted/smoked dietary practices, smoking and alcohol or both addictions are the high-risk factors for GC in a population, and mostly in urban residents. The presenting clinical symptoms among majority of the people are abdominal pain, dyspepsia, weight loss, nausea, epigastric lump and ulcerative growth (Banik et al., 2024).

2.2. Grading and pathological characteristics

GC is a highly heterogeneous disease, both histologically and at molecular level. Histologically, GC is graded as well (Grade 1), moderately (Grade 2) and poorly (Grade 3) differentiated adenocarcinoma (Cretu et al., 2022). Moreover, the molecular characterization of heterogenous GC follows the TCGA and AGCR classifications (Shin et al., 2024; Kim et al., 2022).

The molecular characteristics of early-stage GC involves lymphovascular invasion (LVI), perineural invasion (PNI) and serosal invasion (SI). Presence of lymph node and metastatic lymph nodes impacting nodal status in GC. Moreover, PNI observed in local invasion of GC, pancreatic, biliary tract, and large intestine cancers. Also, presence of pathological SI caused due to direct

invasion or inflammation. In most of the cancers, these invasions predict poor outcome, including useful information for the clinical management of patients with gastric cancer (Yura et al., 2021; Pande et al., 2018).

2.3. GC classification

2.3.1. Borrmann classification

Borrmann classification described the gross or endoscopic findings. It involves Type I polypoid with no ulceration, fungating with ulcerating sharp margin; Type II with elevated borders; Type III ulcerated and diffusely infiltrating cells into surrounding wall; and Type IV flat/diffusely infiltrative or diffusely infiltrating mostly without ulceration appearance (Díaz et al., 2021; Song et al., 2020).

2.3.2. Lauren classification

According to Lauren classification, presence of glandular growth pattern indicates intestinal-type, whereas the absence of such growth signifies diffuse-type GC. Intestinal type GC features glandular epithelial columnar cells, good cellular cohesion and a pushing margin at the invasive edge. The intestinal type typically arises against atrophic gastritis often accompanied by intestinal metaplasia and is more prevalent in older male. In contrast, diffuse type GC consists of scattered, poorly cohesive cells or small clusters of cells with minimal or no gland formation, and a diffuse infiltrative margin. It invades the submucosa early on, with tumor cells frequently spreading within the upper mixed type of gastric adenocarcinoma is also present in few cases (Li et al., 2018; Jimenez at al., 2017).

2.3.3. WHO classification

The present classification of WHO 2010, distinguishes GC into papillary, tubular, mucinous and signet-ring cell subtypes. The poorly cohesive carcinoma type encompasses the Signet ring cell carcinoma (SRCC) with low clinical importance and compromised survival outcome (Machlowska et al., 2020). These histological characteristics presenting molecular characteristics remains

the gold standard for diagnosis and molecular testing overcoming limitations like small sample size. Tumor histology was dominated by adenocarcinoma exhibiting SRC cells which often characterize the diffuse type GC. This feature associate with poor prognosis, treatment resistance, and poor outcome (Sanjeevaiah et al., 2018).

2.4. TNM staging

In AJCC, the T, N, and M categories basically determine the tumor extend, lymph node invasion and spread to distant parts of the body, respectively. The staging can be assigned as clinical staging and pathological/surgical staging. Clinical staging is determined based on physical examinations, biopsies, imaging tests, upper endoscopy or laparoscopy whereas pathological stage is considered based on the spread of cancer cells farther than could be seen on imaging test. The stagings are summarized in the **Table 1** below (In et al., 2017).

Table 1: AJCC TNM staging system for gastric cancer

AJCC stage	Stage grouping	Description
0 *(in situ)*	Tis	Tumor not grown into deeper layers of tissue such as the lamina propria
	N0	Cancer has not spread to nearby lymph nodes
	M0	Cancer has not spread distant parts of the body
IA	T1	Tumor has grown from the mucosa the lamina propria
	N0	Cancer has not spread to nearby lymph nodes
	M0	Cancer has not spread distant parts of the body
IB	T1/T2	Tumor is growing into the lamina propria /muscularis propria layer
	N1/N0	Cancer has spread to 1 to 2 lymph nodes /not spread to nearby lymph nodes
	M0	Cancer has not spread distant parts of the body
IIA	T1/T2/T3	Tumor is growing into the lamina propria /muscularis propria/subserosa layer
	N1/N2/N0	Cancer spread to 1 to 2 / to 3 to 6 / not spread to nearby lymph nodes
	M0	Cancer has not spread distant parts of the body

IIB	T1/T2/T3/T4a	Tumor is growing into the lamina propria /muscularis propria/subserosa/ serosal layer
	N3a/N2/N1/N0	Cancer has spread to 7 to 15/ to 3 to 6/1 to 2/ not spread to nearby lymph nodes
	M0	Cancer has not spread distant parts of the body
IIIA	T2/T3/T4a/T4a/T4b	Tumor is growing into the muscularis propria/subserosa/ serosal layer/ nearby organs or structures
	N3a/N2/N1/N2/N0	Cancer has spread to 7 to 15/ to 3 to 6/1 to 2/ not spread to nearby lymph nodes
	M0	Cancer has not spread distant parts of the body
IIIB	T1/T2/T3/T4a/T4a/T4b/T4b	Tumor is growing into the lamina propria/muscularis propria/subserosa/ serosal layer/ nearby organs or structures
	N3b/N3b/N3a/N3a/N1/N2	Cancer has spread to 16 or more/7 to 15/ to 3 to 6/1 to 2 nearby lymph nodes
	M0	Cancer has not spread distant parts of the body
IIIC	T3/T4a/T4b/T4b	Tumor is growing into the subserosa/ serosal layer/ nearby organs or structures
	N3b/N3b/N3a/N3b	Cancer has spread to 16 or more/7 to 15 nearby lymph nodes
	M0	Cancer has not spread distant parts of the body
IV	Any T	The cancer might or might not have grown into any of the layers of the stomach wall
	Any N	Might or might not have spread to nearby lymph nodes
	M1	The cancer has spread to distant organs such as the liver, lungs, brain, or the peritoneum

2.5. Treatment approaches

2.5.1. Surgical approach

According to Japanese Gastric Cancer Treatment Guidelines 2021 (6th edition) gastric surgery include standard gastrectomy and non-standard gastrectomy. These follows extent of gastric resection, and lymph node dissection. The standard gastrectomy involves resection of two-third of the stomach along with a D2 lymph node dissection. Whereas non-standard gastrectomy comprises modified and extended surgery including D1, D1 +, D2, D2+ etc. lymphadenectomy. The D2 lymphadenectomy is directed for cN + or ≥ cT2 tumors and a D1 or D1 + for cT1N0 tumors (1.5 cm or smaller in diameter). The surgery include total, distal, proximal, local, subtotal and segmental

gastrectomy with curative intent of proximal margin of at least 2 cm for T1, 3 cm for T2, and 5 cm for those with an infiltrative growth pattern. Palliative or reduction surgery are non-curative surgery offered to incurable metastatic patients (liver metastasis and peritoneal metastasis) with symptoms of bleeding or obstructions to maintain good quality of life of patients, improve nutritional intake and good prognosis. (JGCA 2023; Tsekrekos et al., 2024).

2.5.2. Chemotherapy

The current guidelines include various chemotherapy options, mostly pre or perioperative/neoadjuvant and postoperative/adjuvant chemotherapy, practices available for non-metastatic locally advanced GC to reduce relapse and metastasis (Meng et al., 2024). In Western countries, both neoadjuvant and adjuvant chemotherapy considered as the preferred treatments, whereas in Asia, the standard approach is D2 lymphadenectomy followed by postoperative adjuvant chemotherapy. However, perioperative chemotherapy reportedly increases the OS of the patients (Joshi et al., 2021; Tokunaga et al., 2020).

a) Neoadjuvant chemotherapy (NACT)

Patients with potentially resectable clinical T_2N_0 and lymph node dissection are generally advised for pre-perioperative NACT over upfront surgery. NACT can potentially lead to tumor downstaging, improved R0-resection rate, (Joshi et al., 2021; Song et al., 2017). The regimens and their combinations are not standardized around the world with differences between East, West and Asian countries (Sato et al., 2023; Yamashita et al., 2021; Khan et al., 2019).

Initially, epirubicin, cisplatin, and 5-fluorouracil (ECF) as perioperative chemotherapy was widely used. However, regimens like, docetaxel, cisplatin, and 5-fluorouracil (DCF) regimen and 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) regimen have shown efficacy over DCF and ECF, later modified FOLFOX6 with higher doses of leucovorin and 5-fluorouracil apparently achieve significant tumor regression. In some part of the world, including India, 5-fluorouracil, leucovorin, oxaliplatin, and docetaxel (FLOT4) regimen has been used due to its superior outcomes over other regimens (Farrokhi et al., 2022; Panda et al., 2022).

b) Adjuvant chemotherapy

The patients with pT3/T4 lesions or lymph node-positive status in stage II or higher recommended adjuvant chemotherapy. Regimens like capecitabine and oxaliplatin (CAPOX) is suggested in patients who underwent curative-intent gastrectomy with D2 lymphadenectomy. Some countries use oral S-1 (derivative of 5FU) alone, or S-1 plus docetaxel instead of CAPOX. At present, S1, DS, and XELOX are widely reliable adjuvant chemotherapy regimens, however, shown limited overall efficacy (Joshi et al., 2021; Yamashita et al., 2021).

2.6. Multidrug Resistance in GC

Multidrug resistance (MDR) can be inherent or acquired ability of cancer cells to withstand various anticancer drugs while reducing the treatment efficacy. Nearly 90% of chemotherapy cases fails due to drug resistance against many potential anticancer agents, making it difficult to break the bottleneck of survival rate in advanced GC (Emran et al., 2022). The MDR in GC mainly facilitated by enhanced drug efflux, decrease drug uptake, cancer stem cells (CSC), hypoxia, enzymatic activity, epithelial-mesenchymal transition (EMT), altered epigenetics, enhanced xenobiotic metabolism, activation of DNA repair machinery and intracellular detoxification system. Additionally, recently, microRNAs and long noncoding RNAs are found to induced the development of MDR in GC (Liu et al., 2024; Vaidya et al., 2022).

2.6.1. Intrinsic and acquired drug resistance

Based on the time of development of resistance phenotype of the cancer cells, drug resistance can be intrinsic and acquired. Intrinsic resistance exists prior to drug exposure, while acquired resistance develops after therapy. The mechanisms of both resistances can co-exist in tumors during treatment.

The intrinsic resistance mechanisms of cancer cells before drug treatment usually causes reduced responses to the drug treatment. Intrinsic resistance is resultant of inherited genetic alterations, unresponsive subpopulations like cancer stem cells, or activation of intrinsic pathways that eliminate anticancer

drugs. Evidently, GC patients with HER2 overexpression exhibit intrinsic resistance to cisplatin shown to activate the transcription of Snail, which eventually induces EMT. In contrast, HER2/Snail double-positive patients presented an even lower cisplatin response rate than single-positive patients who have received chemotherapy for the first time (Emran et al., 2022; Wang et al., 2019).

Furthermore, acquired resistance refers to the gradual decrease in the competence of anticancer agents after cycles of drug administrations. This type of resistance probably induced by the activation of a second proto-oncogenes, a new driver gene; modification of drug targets; and changes in the tumor microenvironment. An important example is, mutation in BCR-ABL cause resistance against Imatinib, a tyrosine kinase inhibitor targeting BCR-ABL in chronic myeloid leukemia which develops after cycles of chemotherapy (Emran et al., 2022; Wang et al., 2019).

Mechanisms of acquired drug resistance can be totally different from the pre-existing intrinsic drug resistance. It is important to distinguish intrinsic and acquired resistance, however some specific underlying mechanisms are similar.

2.6.2. Current Therapeutic Challenges Posed by MDR in GC

a) Reduced efficacy of standard chemotherapy and high toxicity

Emergence of MDR significantly diminishes the effectiveness of standard chemotherapeutic agents, such as 5-fluorouracil, cisplatin, and paclitaxel in GC (Song et al., 2017; An et al., 2015; Zhang et al., 2010). One key factor contributing to this reduced efficacy is MDR-related gene polymorphism, such as C3435T polymorphism has been linked to clinical outcomes in GC patients undergoing postoperative adjuvant chemotherapy (Li et al., 2011). Additionally, elevated glycolysis and autophagy have been identified to associate with poor overall survival and resistance to FLOT chemotherapy in locally advanced gastric cancer (LAGC). Studies have highlighted that targeting glycolysis, and autophagy could enhance chemosensitivity and improve therapeutic outcomes in LAGC patients (Helal et al., 2024). Emerging targeted therapies, such as pemigatinib, a selective fibroblast growth factor receptor (FGFR) inhibitor,

significantly shown to reverse ABCB1-mediated MDR and metal ion imbalances (Na, K, Mg, Ca, Fe, Cu, Zn, and Mn) that contribute to drug resistance (Zhang et al., 2024; Xiao et al., 2024).

b) Heterogeneity of GC and tumor microenvironment (TME) resistance

Gastric carcinoma exhibits substantial molecular and phenotypic heterogeneity including immunosuppressive microenvironment, while challenging the durable response of chemotherapy (Yasuda et al., 2024). Furthermore, TME, consisting of stromal cells, immune cells, and extracellular matrix components, which actively acts as a barrier to drug penetration thereby promotes chemoresistance by fostering conditions like hypoxia, acidity, and immunosuppression, which enhance tumor cell survival (Hass et al., 2020).

c) Complexity of resistance mechanisms in GC

Anticancer drugs suppress the onset and development of GC, as well as MDR, thereby multiple signalling pathways. Initially, the treatment is responsive, however, cancer cells develop secondary resistance by adopting different strategies (**Figure 1)**. In recent times, some of them became a hotspot in for drug reversal in MDR cancers including GC:

1. **Enhanced efflux pumps:** Studies extensively explored the drug efflux ATP-binding cassette (ABC) family proteins including MDR1, MDR2, MRPs and other. MDR1 also known as P-glycoproteins (P-gP), encoded by ABCB1 gene. P-gP inhibitors were tested in clinical trials for cancer patients; however, none have been approved for clinical use (Weidner et al., 2016).
2. **Alteration of drug targets:** It presents limitations to targeted therapy. Tyrosine kinase inhibitors (TKIs) such as crizotinib, foretinib, and TPX-0022 elzovantinib for MET and KRAS; trastuzumab for HER2; Dovitinib for FGFR2 develops resistance against due to target alterations (Zhang et al., 2024; Dickerson et al., 2024).
3. **Escaping cell death:** Cancer cells combating both accidental cell death (ACD) and regulated cell death (RCD). RCD involving apoptosis, autophagy, and ferroptosis associated with the development of

chemotherapy resistance in GC (Tong et al., 2022; Chen et al., 2021; Xiao et al., 2020).

4. **DNA methylation, RNA modification, and Ubiquitination or deubiquitination** regulate the expression of genes upregulate in response to chemotherapeutic drugs (Liu J et al., 2024).
5. **Non-coding RNA (ncRNA):** The ncRNAs are involved in the development of chemoresistance in GC while regulating tumor-related processes such as migration, EMT, and metastasis (Raei et al., 2021).

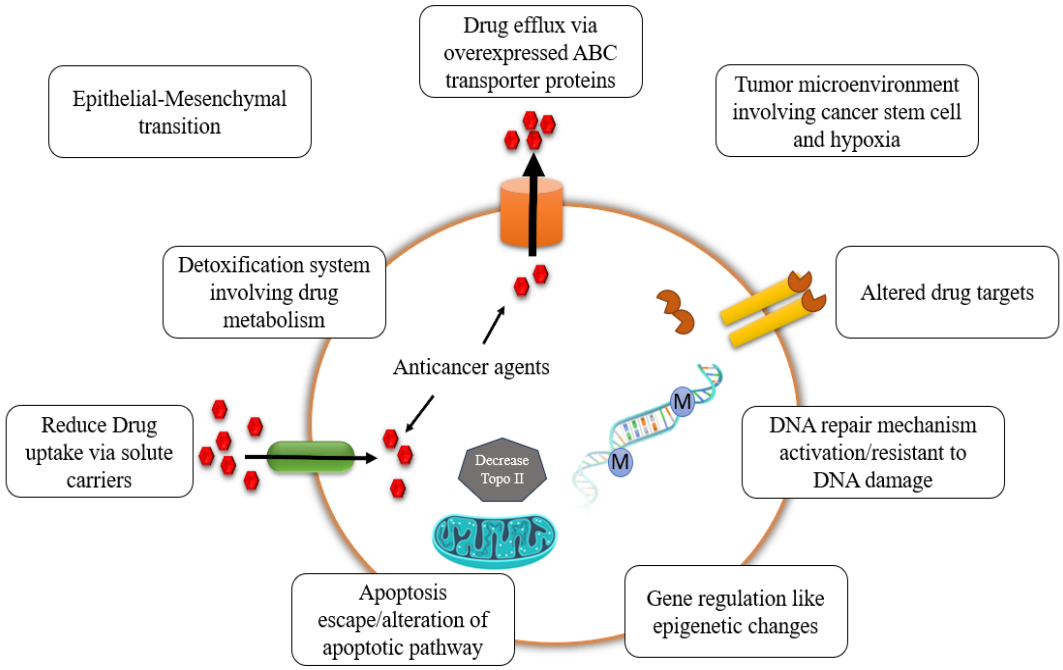


Figure 1: Schematic representation of mechanisms of MDR in GC

2.6.3. P-glycoprotein (P-gP)

P-glycoprotein (P-gP) is one of the hallmarks of MDR, often stands for "permeability glycoprotein" composed of two long transmembrane domain (TMD) of six transmembrane α-helical segments and two cytoplasmic nucleotide binding domains (NBD) (Skinner et al., 2023; You D et al., 2020). It has multiple drug binding sites for agents like etoposide, doxorubicin, cisplatin, 5-Fluorouracil, paclitaxel and vinblastine and others (Gao et al., 2021; Mai et al., 2019). P-gP is found to be epigenetically regulated on xenobiotic exposure (Dong et al., 2023).

a) Role of P-glycoprotein in GC

P-gP has been reported to be overexpressed in a number of resistance tumors, including GC (Tian y et al., 2023). According to studies in GC, elevated levels of lncRNAs such as GHETM1, and differentiation antagonizing non-protein coding RNA (DANCR) are closely associated with upregulation of ABCB1 expression. Moreover, it has been discovered that zinc finger protein 139 inhibits the transcriptional activity of the microRNA (miRNA/miR)-185 promoter, which lowers the amounts of miR-185 and, as a result, lowers the expression of ABCB1. Non-coding RNAs (lncRNAs and miRNAs) are transcribed from the genome and are essential for controlling epigenetics and signal transduction *in vivo* (Biswal et al., 2024; Tan B et al., 2018). P-gP mediated MDR regulated by numerous signalling pathways, including MEK/ERK, PI3K/Akt, NF-κB, Wnt, and STAT3 (Ding et al., 2022; Sui et al., 2021; Ren H et al., 2019) **(Figure 2)**. Targeted inhibitors in clinical treatment were introduced in order to reverse MDR and restore the therapeutic effect of chemotherapy drugs by inhibiting P-gP, in order to alleviate chemotherapy resistance. Some potential P-gP Inhibitors such as verapamil, cyclic peptides (cyclosporin A, PSC833), tamoxifen, sildenafil, curcuminoids, flavonoids, LY335979 (zosuquidar), GF120918 (elacridar), didn't pass the FDA approval for clinical use. Noncoding RNAs, miRNAs and siRNAs can directly or indirectly inhibit P-gP-mediated MDR (Yalamarty et al., 2022; Kang Y et al., 2022; Deng Xj et al., 2021).

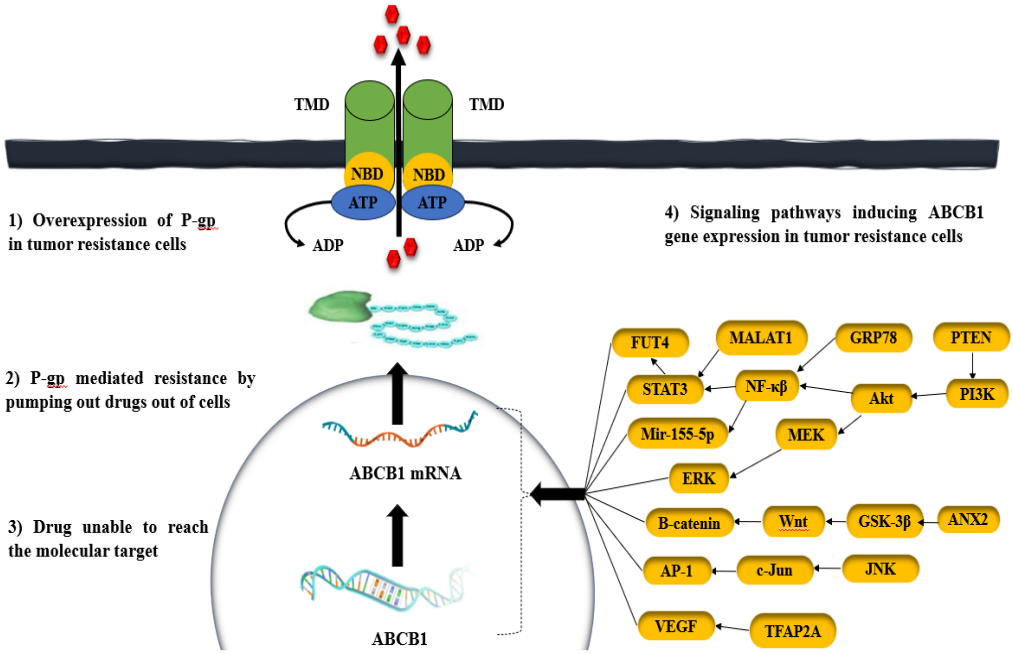


Figure 2: Illustration depicts the structure, function and regulation of ABC transporters in multidrug resistance cancer.

b) Limited availability of predictive biomarkers

The lack of reliable biomarkers for detecting MDR early or monitoring therapeutic responses hampers personalized treatment strategies. Identifying biomarkers could help predict resistance and guide targeted therapies (Matsuoka et al., 2018). Traditional tissue-based, liquid, methylation, immune-therapy biomarkers have shown reliance stratifying GC into subtypes, however challenged by tumor heterogeneity (Ma et al., 2024). Therefore. There is an unmet need for identifying novel targets for MDR in GC.

2.7. Soluble Resistance Related Calcium Binding Protein (Sorcin): molecular characteristics and biological Role

Sorcin is a globular calcium activated oncogenic protein, encoded by the SRI gene, of distinct isoforms (A, B, C, D, and X1) (Genovese et al., 2018). Sorcin is primarily localized in cytoplasm and translocate to sarcoplasmic reticulum, mitochondria, nucleus and plasma membrane, in response to calcium elevation (Genovese et al., 2020). In cancer, Sorcin is emerging as potential contributor, for its intricate role in cancer progression and chemoresistance. It is observed to

be the key contributor of mechanisms like initiation, progression, metastasis, and survival in cancers (Shabnam et al., 2018; Lalioti et al., 2014).

2.7.1. Structure, and Ca^{2+} -mediated activation of Sorcin

Sorcin is an allosteric protein of 198 amino acids long characterized by N-terminal and C-terminal domains (Saravanan et al., 2023). The N-terminal is highly variable segment, rich in glycine, proline, and hydrophobic residues, and carries a short, flexible N-terminal domain (NTD). In contrast, the C-terminus contains a Ca2+-binding domain known as the Sorcin calcium binding domain (SCBD) (Xie X et al., 2001). Secondary structure of Sorcin is a dimer consisting of helix-loop-helix motif of eight alpha helices connected by loops namely D/G-helix. The helices form five EF-hands (EF1-EF5) which looped with each other through short beta-sheets at SCBD complex. In SCBD complex of one Sorcin monomer, EF1 pairs with EF2 and EF3 with EF4. The unpaired EF5 pairs with the free EF5 hand of corresponding Sorcin monomer to bring about homodimerization. In rare cases, EF5 also binds EF4 hand to form heterodimer. EF1 is highly conserved and have high affinity Ca2+ binding sites when pairs with EF2 followed by EF3. On Ca2+ binding, the D-helix of the EF1-EF2 pair now connects EF2 with EF3 which frees the EF1 hand, exposing the hydrophobic surfaces (increases hydrophobicity). On the other hand, the G-helix connects EF4 to the EF5. The binding brings out the conformational change and alters the inactive closed form of the protein to activated open form (Zhou X et al., 2019). The open form of the protein exposes the hydrophobic residues of D-helix, decreasing solubility of Sorcin in cytoplasm and its translocation in different target sites (Colotti G et al., 2014). Sorcin forms tetramers at a slightly acidic pH and a stable dimer at a neutral pH. Interestingly, Sorcin is the only PEF protein that contains potential phosphorylation sites at serine and threonine residues of EF4 and EF5 C-terminal domain (**Figure 3).**

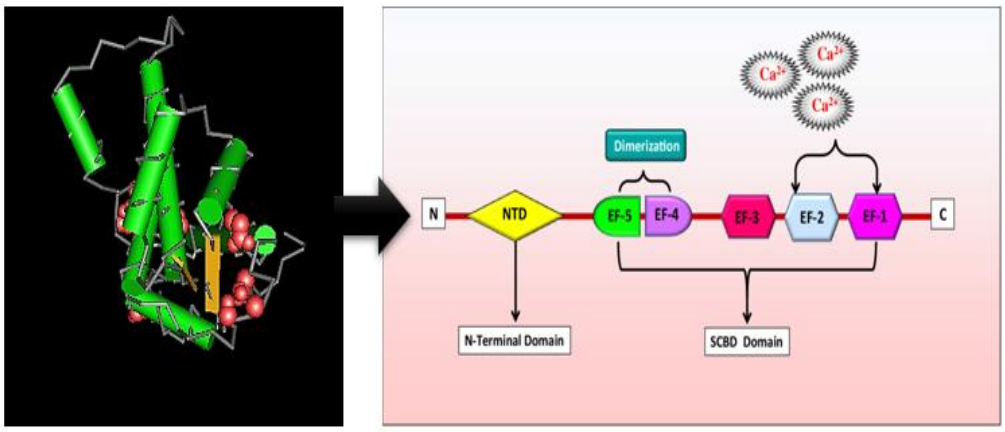


Figure 3: Crystalline and schematic representation of Sorcin (Shabnam et al., 20218).

2.7.2. Sorcin regulating calcium homeostasis

Sorcin acts as a calcium buffer, sequestering calcium ions on cytosolic calcium elevation and releasing them during calcium depletion (Xie X et al., 2001). Reportedly, Sorcin modulates various calcium transporters and channels including plasma membrane calcium ATPases (PMCAs), inositol 1,4,5-trisphosphate receptors (IP3Rs), and sarco/endoplasmic reticulum calcium ATPases (SERCAs), governing calcium uptake, storage and its release into the cytosol from endoplasmic reticulum (ER) (Zhou et al., 2019; Aracena P et al., 2013). Sorcin enhances the activity of SERCA and interacts with inositol 1,4,5-trisphosphate receptors (IP3Rs) to attenuate calcium release from ER (Lokuta et al., 1997; Woll et al., 2022). Furthermore, it physically interacts with the ryanodine receptor (RyR) in ER to inhibit RyR2 activity, thereby reducing the release of calcium from the ER (Colotti G et al., 2014; Woll et al., 2022). The reduced calcium release from the ER, prevents calcium overload to escape apoptosis.

2.7.3. Sorcin in cancer

Sorcin is differentially expressed across various cancers, including glioblastoma multiforme, nasopharyngeal, breast, lung, gastric, gallbladder, colorectal, hepatocellular, bladder, ovarian, as well as leukemia and myeloma, as demonstrated in various tumor models (Jain et al., 2023; Ling et al., 2021; Zhang et al., 2019; Tuo et al., 2017; Dabaghi et al., 2016; Gao et al., 2015; Liu

et al., 2014; Gong et al., 2014; Yokota et al., 2006). Furthermore, Sorcin expression regulates mechanisms that encompasses aggressive clinical features and distinct stages of tumor development including initiation, progression, epithelial mesenchymal transition (EMT), migration, invasion, angiogenesis, metastasis and metabolism while inhibiting programmed cell death (Liu et al., 2023; Gupta et al., 2018; Tuo et al., 2017; Tong et al., 2015Hu et al., 2014).

2.7.4. Mechanism of Sorcin in cell cycle

Evidently, Sorcin interacts cell cycle proteins, such as AURKA/B, PLK-1, SYL1, ANXA11, PSRC1, CACNB1, among others during mitosis and cytokinesis (Chen et al., 2009; Skop et al., 2004). Mechanistically, Sorcin suppression reduce the expression of p21, cyclin D1, c-Myc, and phospho-Src, while activating p53, leading to cell cycle arrest in both the G0/G1 and G2/M phases (Xu et al., 2015; Zhao et al., 2010).

During cell division, Sorcin participates in phosphorylation-dephosphorylation network important for mitosis and cytokinesis. Moreover, Sorcin localizes in the nucleus during interphase. While in prophase and metaphase, Sorcin-containing vesicles accumulate in the apical region of the mitotic spindle. During anaphase, these vesicles concentrate in the central zone of the spindle, and by late telophase, they predominantly translocate in the midbody. Where, Sorcin interacts with Aurora A, Aurora B, and Polo-like kinase 1 (PLK1) in the cleavage furrow in the midbody, facilitating successful cytokinesis (Ghosh et. al., 2024) **(Figure 4**).

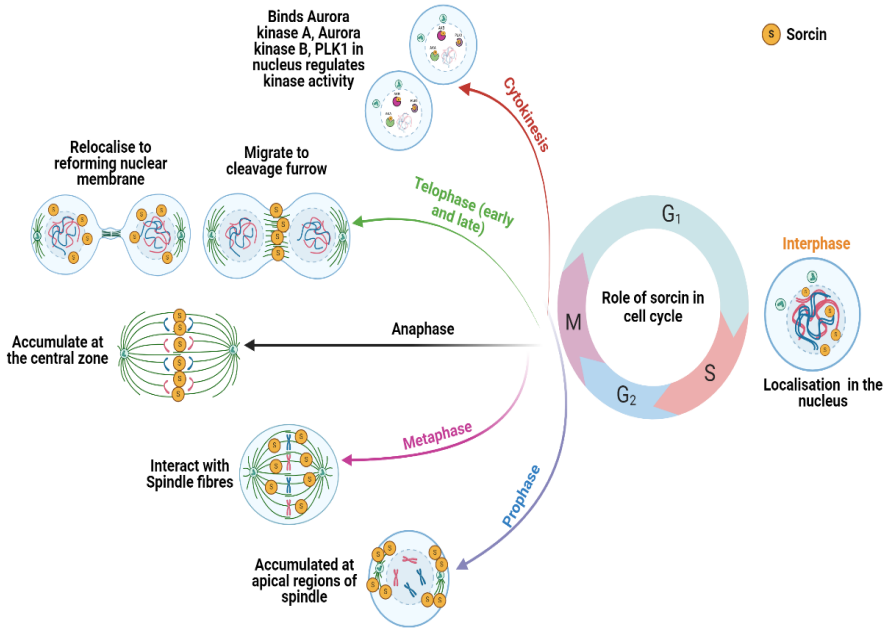


Figure 4: Role of Sorcin in various phases of the cell cycle and its interaction with AURKA/B and PLK1 (Ghosh et al., 2024).

2.7.5. Role of Sorcin in tumor progression, metastasis and metabolism

Sorcin activate key signalling pathways such as EGFR/PI3K/AKT and RAS/RAF/ERK, thereby modulating transcription factors like MYC and FOXO to stimulate proliferation and survival (Tuo et al., 2017; Gao et al., 2015). It interacts with ANXN7 by downregulating N-cadherin, vimentin, and α-SMA levels and upregulating N-cadherin, facilitating EMT, migration and invasion (Titi et al., 2024; hu et al., 2014). Furthermore, Sorcin binds to STAT3, activating MMP2/9 and CTSZ, further supporting metastasis (Tuo et al., 2017). In angiogenesis, Sorcin reportedly regulate VEGF signalling to promote endothelial cell proliferation via nitric oxide synthase (Gupta et al., 2018). It also controls glucose tolerance by regulating cytosolic flux and ER Ca^{2+} storage, via NFAT and ATF6 transcriptional activity (Parks et al., 2021; Marmugi et al., 2016; Noordeen et al., 2012). Sorcin further impacts the tumor microenvironment where it promotes immune cell infiltration and immune evasion and maintain cancer stemness through various stemness markers (CD133, CD44, CD24, SOX2 and ALDH1) (Zhang et al., 2024). Furthermore, Sorcin amplifies P-gP expression, thereby enhancing drug efflux leading to drug resistance **(Figure 5).**

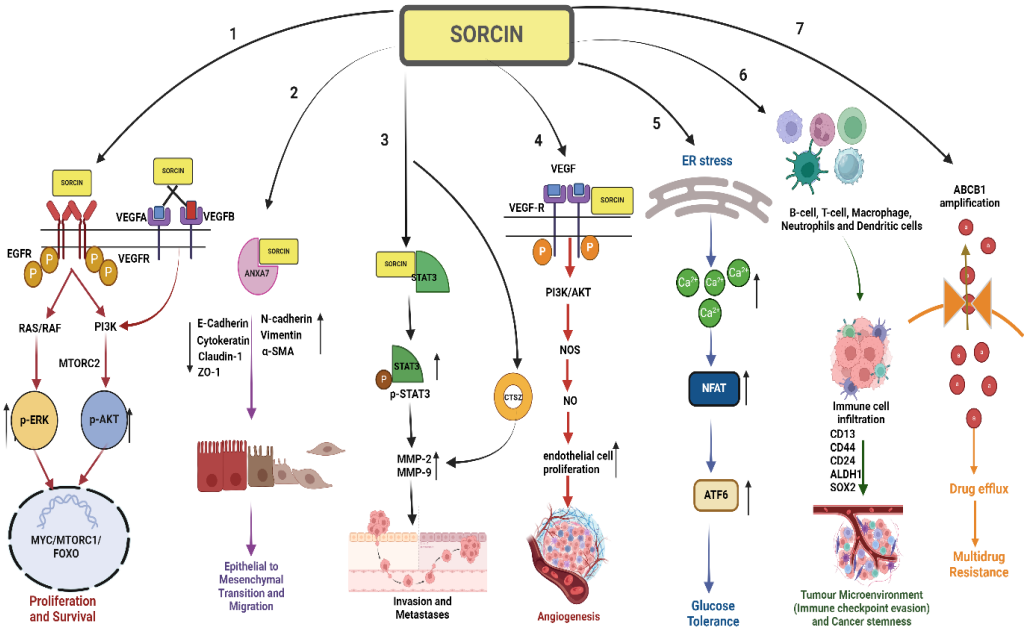


Figure 5: Multifaceted role of Sorcin in various hallmarks of cancer. ↑= Upregulation; ↓=Downregulation. (Ghosh et al., 2024).

2.7.6. Mechanism of Sorcin in programmed cell death

Sorcin employs distinct mechanisms to modulate different signaling pathways, imparting its protective effect against cell death **(Figure 6)**.

In mitochondria-mediated apoptosis -

(a) Sorcin binds to STAT3 to activate CaMKII, and facilitate the NF-κB pathway, leading to the transcription of Bcl2 and IAP via the p50-p65 complex, thereby inhibiting apoptosis (Li et al., 2024; Anthony et al., 2007).

(b) It also maintains calcium homeostasis and alleviates ER stress in cancer cells, with the help of Fli1by inhibiting RyR and inducing SERCA. Additionally, Sorcin activates Bip/GRP78, which inhibits Caspase-12, further preventing apoptosis (Choi et al., 2020).

(c) Sorcin further binds to TRAP1, inhibiting pro-apoptotic proteins such as Bax, Caspase-3, and PARP, thus providing protection against apoptosis (Maddalena et al., 2011; Landriscina et al., 2010).

(d) Additionally, it interacts with PDCD6, impeding mitochondria-mediated apoptosis in both drug-sensitive and drug-resistant cancers (Sun et al., 2018; Matassa et al., 2013).

(e) Sorcin activates ERK/AKT signalling, which leads to the phosphorylation of CREB 1 in MDR cells. This in turn promotes P-gP transcription, reducing intracellular drug concentrations and thus inhibiting apoptosis in MDR cancers (Yamagishi et al., 2014).

In Pyroptosis Regulation-

(f) Sorcin binds to the inflammasome, inhibiting caspase-1 activity, thereby regulating pyroptosis (Li Z et al., 2024).

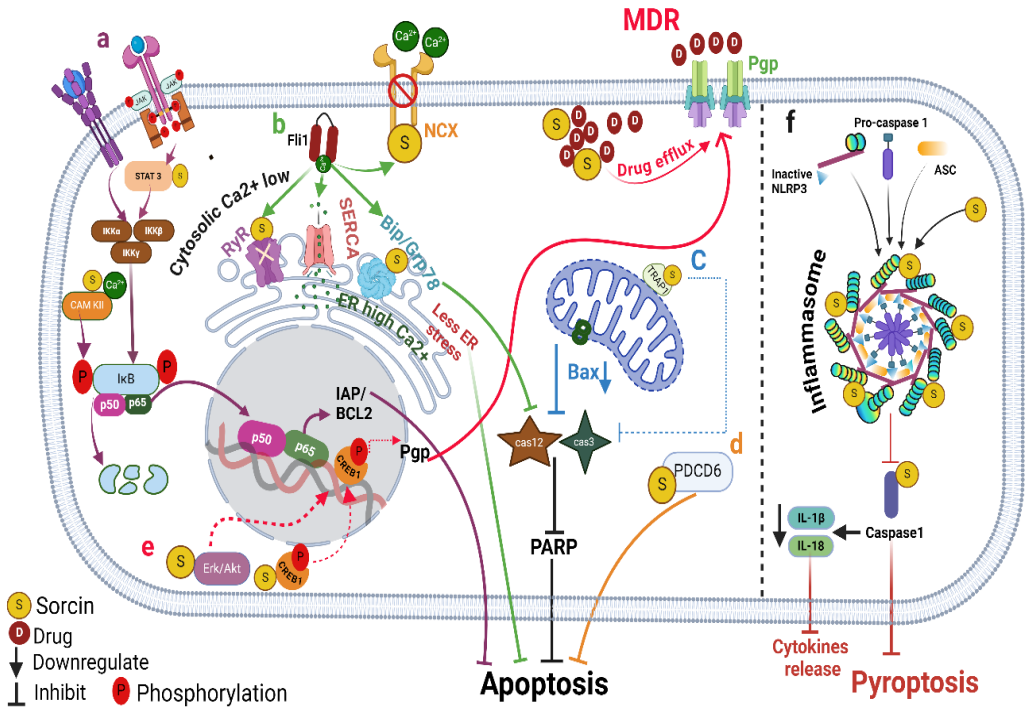


Figure 6: Sorcin regulating programmed cell death events. (Ghosh et al., 2024).

2.7.7. Sorcin in intrinsic and acquired MDR

Sorcin binds therapeutic drugs, including gemcitabine, vinblastine, vincristine, doxorubicin, daunorubicin, Adriamycin, Irinotecan, 5-fluouracil, platins, and taxanes, with high affinity, thereby enhancing resistance against these xenobiotics during cancer treatment (Genovese et al., 2017; Qu et al., 2010)

(Table 2). Sorcin elevation could results from genetic mutations, epigenetic changes or conserved molecular mechanism in resistant cancer cells. Furthermore, it regulates calcium homeostasis differently in resistant cells, contributing to the broader context of drug resistance further enhancing overall drug resistance (Wang et al., 2022; Demidova et al., 1995; Bouchelouche et al., 1991). Previously, Sorcin amplification did not shown to possess collateral sensitivity or any cross resistance to chemotherapeutic drugs (Yamagishi et al., 2014). In contrast, its action against mono-therapeutic agent deliberates cross resistance to other chemotherapeutic drugs along with the up-regulation of P-gP in various cancers. This phenomenon, addresses the development of secondary resistance often considered as acquired resistance (Qi et al., 2006). However, the concrete difference between intrinsic and acquired resistance in context of Sorcin is still unclear in the literature.

Table 2: Overexpression of Sorcin in drug resistant cancer cells in response to chemotherapeutic drugs (Ghosh et al., 2024)

Sl. No	Chemotherapeutic drugs	Cancer	Tumor model	References
1	Gemcitabine	Non-small cell lung cancer (NSCLC); Gemcitabine NSCLC	H460 cells; H460/ GEM	Qu et al., 2010
2	Vincristine	Human Gastric Cancer; Vincristine resistant gastric cancer	SGC790; SGC790/VCR	Yamagishi et al., 2014; He at al., 2011; He et al., 2008; Li et al., 2008; Yi et al., 2006; Wang et al., 1995
		Leukaemia cells; Adriamycin resistant leukaemia cells	K562; K562/A02	
		Vincristine resistant leukaemia cells	ARH77 Vin^R	
			U937 Vin^R	
		Ehrlich ascites cancer cells	EHR/VCR+	
		Hamster lung cancer cells; vincristine-resistant hamster lung cancer cells	DC-3F; DC-3F/VCRd-5L	
		Colchicine resistant Chinese hamster ovary cells	CH^RC5	
		Chinese hamster ovary cells	AuxB1 cell line fractionated X-irradiation generated sublines	

			designated DXR-10	
		Vincristine-resistant lymphoma cells	HOB1; HOB1/VCR1.0	
3	Daunorubicin	Ehrlich ascites cancer cells	EHR2/DNR+	Zhang et al., 2021; Bouchelouchee et al., 1991
		Adriamycin resistant leukaemia cells	K562/A02	
4	Actinomycin D	NA	QUA/ADj	Wang et al., 2021
		The Chinese Hamster Ovary cell line	hamster CHO subline	
5	Methotrexate	Human methotrexate leukaemia cell line	HL60 MTX^R	Liu et al., 2023; Lee et al., 1996;
			U973 MTX^R	
		Methotrexate T lymphocyte cell line	Jurkat MTX^R	
		Leukemia	CCRF-CEM/MTX	
6	Adriamycin	Gastric Cancer; Vincristine resistant gastric cancer	SGC790; SGC790/VCR	Zhang et al., 2021; Wang et al., 2021; Liu et al., 2014
		Leukaemia cells; Adriamycin resistant leukaemia cells	K562; K562/A02	
		Neuroblast cell	BE (2)-C/ADR	
7	Doxorubicin	Lung carcinoma cells	H1299; Calu-1; A459	Genovese et al., 2017
		Adriamycin resistant leukaemia cells	K562/AD2	
		Breast cancer cell	MDA-MB-468; MDA-MB-231	
		Adriamycin breast cancer cell	MCF7/AD2	
		Chinese hamster ovary cells	AuxB1	
		Doxorubicin resistant leukaemia cells	ARHD60 DXR	
8	Cisplatin	Leukaemia cells; Adriamycin resistant leukaemia cells	A549/DDP	Genovese et al., 2017; Altharawi et al., 2021; Goncharova et al., 2000
		Cisplatin resistant leukaemia cell line	HL60 $cisPt^R$	
		Cisplatin resistant T lymphocyte cell line	Jurkat $cisPt^R$	
		Cisplatin resistant non-Hodgkin's B-cell line	DoHH-2 $cisPt^R$	
		Leukemia	Murine P388 subline	
		Djungarian hamster cell line	DM15 subline	
9	Oxaliplatin	Human colorectal cancer	Human HT-29 and HCT-116	Maddalena et al., 2011
10	Paclitaxel	Ovarian cancer cells; platinum resistant ovarian cancer cells	OVCAR 3; OVCAR3/PTX	Wang et al., 2022;

		Human Gastric Cancer; Vincristine resistant gastric cancer	SGC790; SGC790/VCR	Zhang j et al., 2021
11	5-Fluorouracil (5FU)	Human Gastric Cancer; Vincristine resistant gastric cancer	SGC790; SGC790/VCR	Maddalena et al., 2011
		Human colorectal cancer	Human HT-29 and HCT-116	
12	Irinotecan	Human colorectal cancer	Human HT-29 and HCT-116	Maddalena et al., 2011
13	Etoposide	Leukaemia cells; Adriamycin resistant leukaemia cells	K562; K562/A02	Maxwell et al., 2011; Koch et al., 1990
		Chinese hamster ovary cells	AuxB1 cell line fractionated X-irradiation generated sublines designated DXR-10	
14	Homoharringtonine	Leukaemia cells; Adriamycin resistant leukaemia cells	K562; K562/A02	Genovese et al., 2017
15	CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone)	Diffuse large B-cell lymphoma (DLBCL)	Patients (Human model); CHOP resistant DLBCL cells	Maxwell et al., 2011;
16	FLOT (5FU/leucovorin; Oxaliplatin; Docetaxel)	Advanced Gastric cancer	Patients (Human model)	(Ghosh et. al., 2024)

2.7.8 Mechanism of Sorcin in the development of MDR

Sorcin is a key contributor of MDR in cancers **(Table 1)**. However, its behaviour varies across different drug-resistant cancers, exhibiting distinct responses to various drugs. Studies have reported that in the onset of MDR, Sorcin was found to localize predominantly in the cytosol in response to doxorubicin while reducing its accumulation in other organelles. Mechanistically, it binds doxorubicin with high affinity in the cytosol, preventing its entry into the nucleus, and facilitates its efflux through the P-gP transporter pump **(Figure 5)**. This reduces intracellular accumulation of doxorubicin and inhibits its ability to mediate genotoxic topoisomerase II- DNA damage, free-radical generation, disruption of mitochondrial membrane potential, and PARP/Caspase pathway. Additionally, it's actions prevent mitotic catastrophe and cellular senescence

(Genovese et al., 2017). In response to etoposide, overexpressed Sorcin modulates the expressions of P-gP, Bcl2 and Bax, hence protecting the cell from drug-induced apoptosis (Hu et al., 2013). Mechanistically, it escalates the promotor activity of the MDR1 gene, by binding between 716 and 709 bp of CRE site, upstream of the MDR1 promoter. Meanwhile, it promotes the phosphorylation of the CREB1 via PKA activation enhancing P-gP expression favouring drug efflux (Lokuta et al., 1997). Moreover, Sorcin controls P-gP expression through ERK/Akt signalling underscoring its unidirectional effect on P-gP regulation in drug resistant cancer cells (Genovese et al., 2017; Maddalena et al., 2011) (**Figure 6e).** In contrast, downregulation of Sorcin, elevates E-cadherin while depleting ZEB-1, VEGF levels to inhibit, migration, invasion and angiogenesis thereby reversing adriamycin resistance (Zamparelli et al., 2010).

2.8. ERK1/2 signalling

Extracellular-signal-regulated kinases1and 2 (ERK1/2) is one of the important pleiotropic kinases of MAPK (mitogen activated protein kinase) cascade which phosphorylate array of downstream proteins or transcription factors. The ERK family are highly conserved serine/threonine protein kinases involving five subgroups: ERK1 through ERK5. Among them, ERK1 and ERK2 are broadly studied and are generally mentioned as ERK1/2 due to their 90% homology. ERK1/2 is a central downstream molecule in transmitting signals from surface receptors to the nucleus. The ERK pathway involves three upstream MAPKKKs including A-RAF, B-RAF, and RAF-1/C-RAF kinases, MAPK/ERK1/2 kinase (MEK) as a MAPKK, while ERK1/2 as MAPK **(Figure 7)**. Activated ERK1/2 phosphorylates the target molecule including transcription factors either in the cytoplasm or nucleus to activate the expression of specific proteins that regulates different cellular processes including proliferation, differentiation, stress response and apoptosis (Kong et al., 2019).

ERK1/2 is generally located in the cytoplasm while on activation either remains in the cytoplasm or translocate to the nucleus. There are multiple ways to activate ERK/MAPK signalling such as: (a) G protein-coupled receptor activation; (b) activation of receptor tyrosine kinase Ras; (c) integrin- mediated

and (d) PKC-mediated activation (Yang S 2017; Roskoski et al., 2012). The ligand-receptor activation at plasma membrane activates the kinase activity of Ras-GTP followed by Raf and MEK. Subsequently, activated MEK directly interacts with the N-terminal region of ERKs, thereby catalyses the bispecific phosphorylation of Tyr and Thr residues in the 'TEY box' of ERK in the cytoplasm. Cytoplasmic phosphorylated ERK1/2 undergo dimerization and translocation to the nucleus. In nucleus, it Phosphorylates nuclear transcription factors like c-Fos, c-Jun, Elk-1, c-Myc, and ATF2 thus influencing cell proliferation, autophagy, oxidative stress, inflammation, cell cycle, growth, differentiation, apoptosis, and tumorigenesis (Ullah et al., 2022; Song et al., 2022; Liu et al., 2018).

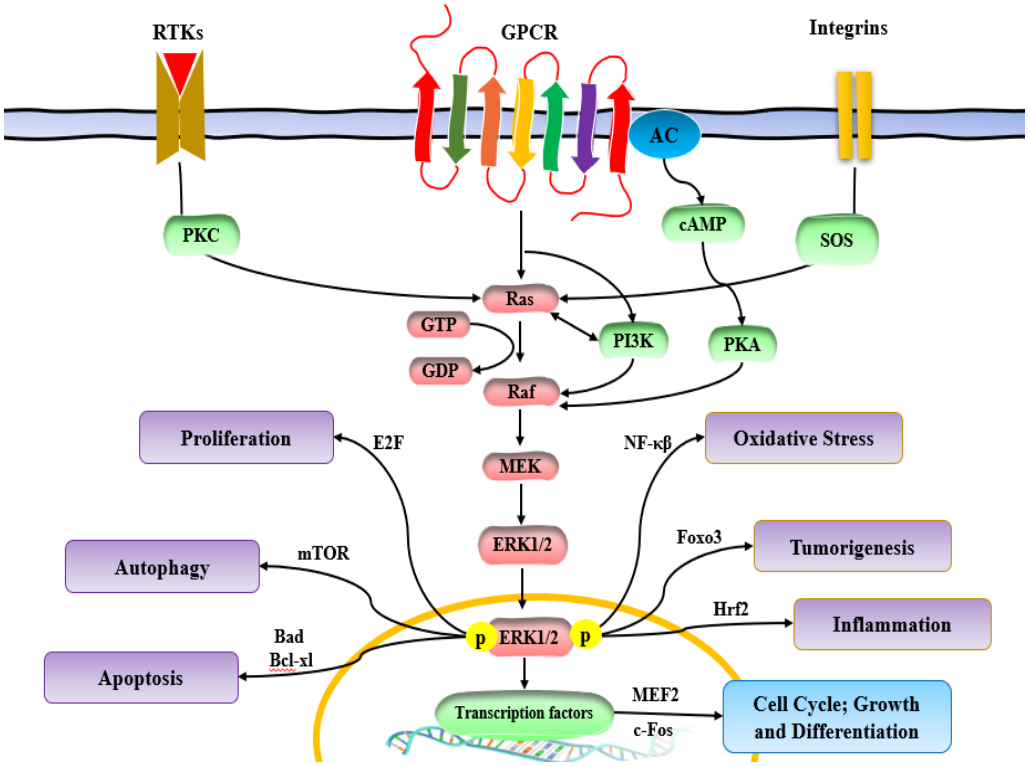


Figure 7: ERK1/2 signalling regulating different cellular processes in cell.

2.8.1. ERK1/2 cascade in GC

In gastric cancer, *H. pylori* infection induces RAS/RAF/ERK1/2 pathway activation in gastric-epithelial cells. Where growth factor receptor-bound protein 2 (GRB2) undergoes phosphorylation in the Src homology 2 (SH2) domain and activates Ras-GTP via the SOS system. However, Ras mutation is

the most frequent oncogene in GC which transform epithelial cells into neoplastic cells through series of cellular changes (Morgos et al., 2024). ERK1/2 promote proliferation and metastasis in GC by recruiting markers like MMP-2/9 (Wang Z et al., 2017). Additionally, ERK1/2 signalling contributes to the development of a multidrug-resistant phenotype by up-regulating anti-apoptotic proteins of the Bcl-2 family and P-gP (Jiang et al., 2018).

Furthermore, *H. pylori* released a protein CagA which induces the ERK1/2 activity, potentially independent of Ras activation (Zhu et al., 2005). Downregulation of chemical factors like gastrin impedes the ERK-P65-miR23a/27a/24 axis leading to excessive growth in GC. The ERK-P65-miR23a/27a/24 axis demonstrated negative association with the effectiveness of FLOT treatment resulting into poor prognosis in GC. Therefore, elevated ERK2 levels in GC patients correlate with promotion or inhibition of gastric cell migration and invasion (Wang Y et al., 2023).

2.8.2. ERK1/2 signalling in multidrug resistance in GC

MAPK/ERK pathway largely implicated in GC progression and drug resistance, thereby challenging chemotherapy. Interestingly, most of the anticancer drugs reduce chemoresistance by downregulating ERK1/2 activity in GC. Particularly, ERK mutations, such as ERK2 E320K and ERK1 R84H contribute to drug resistance. Chemoresistance is closely linked CHD4 upregulation which reduces intracellular cisplatin concentrations through ERK1/2 and MEK1/2 signalling (Wu J et al., 2023). Furthermore, in 5FU-resistant GC, cancer stem cells (CSCs) enter quiescence through p38-MAPK/ c-Myc and epigenetic signaling following chemotherapy (Bou et al., 2023). Furthermore, Ras/ERK1/2 signaling enhances the transcription of P-gP in adriamycin, vincristine, and PTX-resistant GC (Zhao Y et al., 2015).

A number of ERK1/2 inhibitors were tested in clinical trials however, their efficacy faces limitation in MDR cancers. ERK inhibitors works either by targeting the inhibition of its catalytic activity or both catalytic activity and phosphorylation activity at the T-E-Y motif. In drug resistance tumors, the

ERK1/2 inhibitors block the activity of cytoplasmic ERK1/2 stimulating its negative feedback loop to reduce overactivation of upstream cascade components, supporting resistance development. An inhibitor, EPE peptide, specifically block nuclear translocation of ERK1/2, while preserving its cytoplasmic functions (Pan X et al., 2021; Martin-vega et al., 2023). In resistance cells, however, ERK1/2 inhibitors face significant challenges due to the activation of compensatory pathways, usually, PI3K/AKT, challenging their drug delivery efficiency and pharmacokinetic ability.

2.8.3. ERK1/2 and Sorcin

Studies have highlighted the possible mechanism of action of Sorcin through ERK1/2 signalling in cancer. In this context, Soluble factors (G-CSF; Sorcin) found to involve in the ERK-induced up-regulation of ABC transporters (Zhau et al., 2015). Additionally, miR-20a down-regulates EGFR-dependent MEK/ERK and PI3K/AKT pathways to restore chemosensitivity (Zhao et al., 2016). Reportedly, Sorcin regulates ERK1/2 to promote cell proliferation, EMT, migration, invasion, and chemotherapy resistance. A study demonstrated that Sorcin and EGFR expression are correlated and associated with reduced overall survival in cancer patients, where it directly binds to EGFR in a calcium-dependent manner, regulates calcium homeostasis related to EGF-dependent EGFR signalling, and controls EGFR proteostasis and signalling by increasing its phosphorylation. This enhances EGF-dependent migration and invasion (Tito et al., 2023). The interplay between signalling pathways and transporter proteins highlights the complexity of chemoresistance mechanisms underscoring the need of exploring these pathways to enhance therapeutic efficacy in cancers, particularly in GC.

2.8. Research gap

Although there have been considerable literatures explored the role of Sorcin in MDR, there are still important clinical and molecular gaps that are missing. Clinically, it is evident that poor prognosis and chemoresistance are associated with high Sorcin expression, but it has not yet been validated as a reliable biomarker to predict MDR in cancer patients. There is a lack of clinical studies that correlates Sorcin expression with treatment outcomes, patient survival, and therapeutic response, particularly in GC, where MDR remains a major challenge. Additionally, the mechanism by which Sorcin contributes to MDR and tumor progression is still not well understood. A critical gap in current research is understanding the interplay between Sorcin, P-gP, and ERK1/2, particularly the mechanistic insights into how Sorcin regulates ERK1/2 activation and P-gP to drive MDR. Addressing this gap through thoughtful integration of clinical and molecular evaluations, a more effective approach to predict chemotherapy response and validate the mechanistic involvement of Sorcin in MDR and may become achievable.

2.9. Rationale

Sorcin has the potential to predict the MDR phenotype among GC patients. Its molecular association with ERK1/2 in patients receiving neoadjuvant FLOT chemotherapy suggests a possible role in the development of MDR in GC. Furthermore, understanding the underlying mechanistic association of Sorcin with P-gP and ERK1/2 could provide critical insights into its contribution to MDR, contributing to the development of targeted therapeutic strategies.

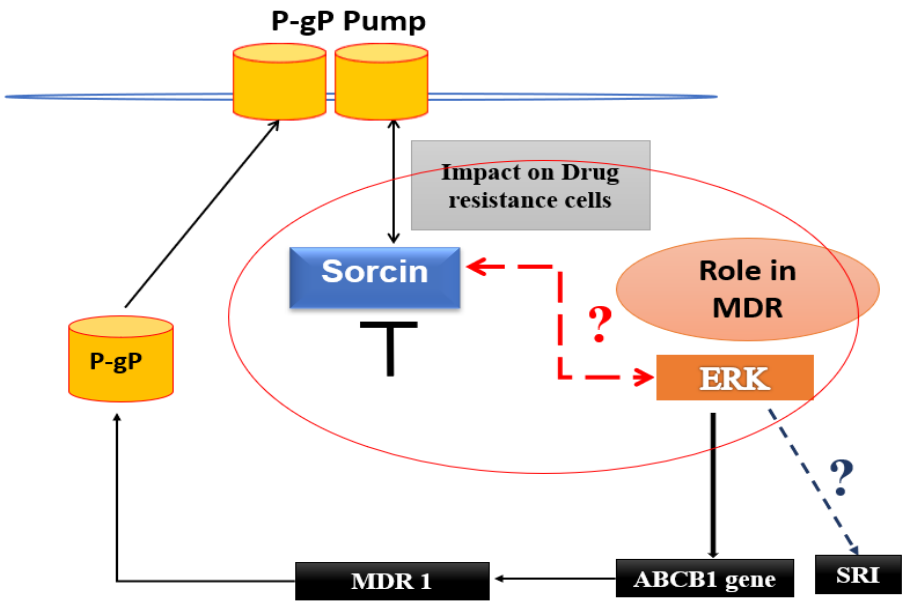


Figure 8: Schematic diagram of the proposed hypothesis to study the underpinning mechanism of Sorcin in GC.

2.10. Aims and objectives

The present work focuses on the role of Sorcin as a biomarker for identifying the MDR phenotype in GC patients and to explore its underpinning interplay with ERK signalling and regulation of P-gP in MDR. Thus, the objectives are:

1. To assess the histopathological and clinicopathological features of advanced gastric cancer in relation to overall survival of patients.
2. To determine the clinical significance of Sorcin expression and its relationship with P-gP and ERK1/2 in advanced gastric cancer patients.
3. To evaluate the molecular interplay of Sorcin, P-gP and ERK1/2 in AGS and drug resistant AGS cell line.
4. Analysis of data for prediction and clinical management of advanced gastric cancer patients.

CHAPTER 3
MATERIALS AND METHODS

3.1. Clinical analysis

3.1.1. Study design and patient enrolment

This prospective study included 78 newly diagnosed non-metastatic gastric cancer patients (stage T2-T4a). Sample and data collection was carried out between August 2019 and September 2021, with follow-up conducted from October 2021 to December 2024. Forty-three eligible participants were recommended for upfront surgery (US) followed by adjuvant chemotherapy (ACT) hold at least 6 cycles and 23 patients underwent neo-adjuvant chemotherapy (NACT) followed by surgery. Patients were administered with 4 cycles of a FLOT regimen composed of a 2-hour infusion of 5-Fluouracil (500–2500 mg/m2), Leucovorin (200 mg/m2), Oxaliplatin (85 mg/m2), and docetaxel (60 mg/m2) as first-line chemotherapy. Treatment was repeated every 2 weeks until disease progression, patient refusal, or unacceptable adverse reactions (Wang et.al., 2019).

The clinical analyses were carried out in collaboration with the Departments of Surgical Oncology, Chemotherapy, and Pathology at Chittaranjan National Cancer Institute (CNCI), Kolkata, India. Following radiological evaluations (MRI, CECT, PETCT), the operable patients were further monitored, and the post-operative tissue biopsies were subjected to molecular analyses: qRT-PCR, Immunohistochemistry (IHC), Western blot, Flow Cytometry, Co-immunoprecipitation (Co-IP), and MTT assays to assess Sorcin, ERK, and P-gP expressions and their interactions. Additionally, their mechanistic activities were performed *in vitro* (**Figure 9**).

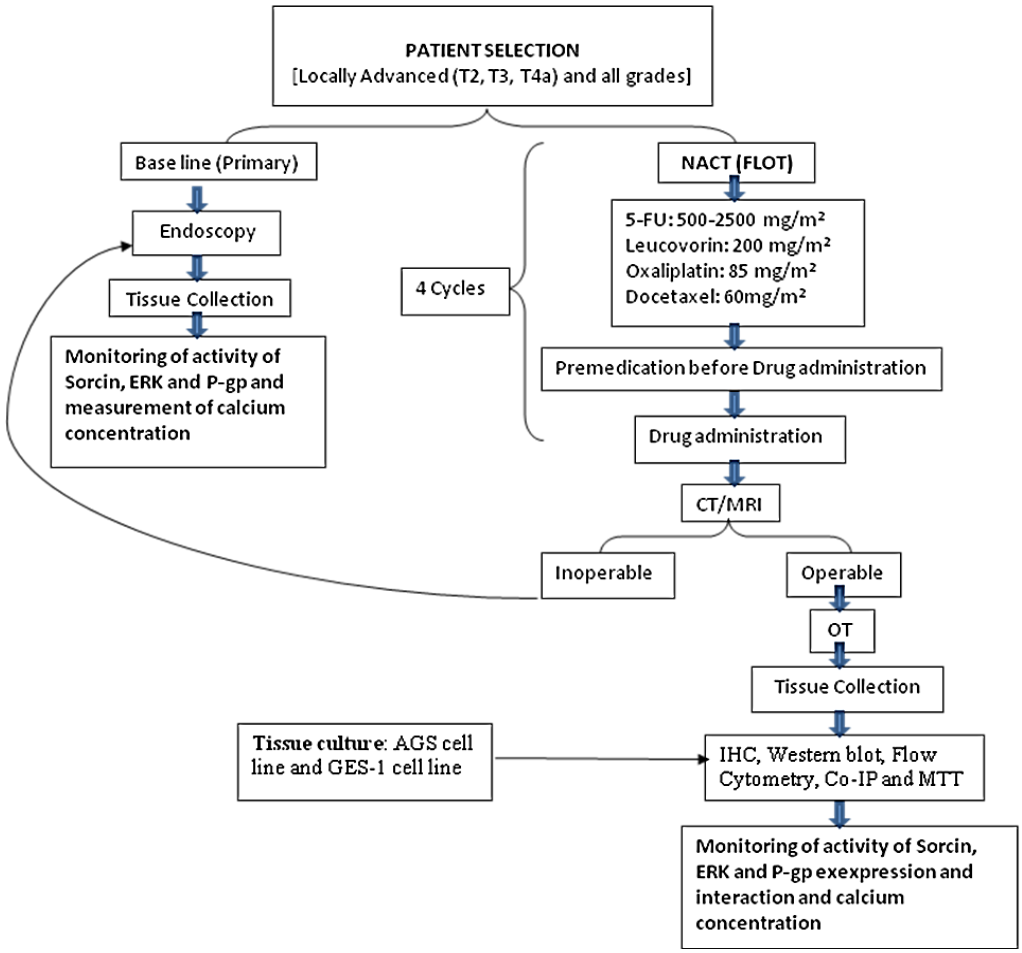


Figure 9: Flowchart representing clinical study design

3.1.2. Inclusion criteria

1. Patients of age ≥18 year newly diagnosed with stage I-IV gastric cancer, including adenocarcinoma of the gastro-esophageal junction.
2. Patient yet to receive chemotherapy according to NCCN guidelines.
3. The histological features of the primary tumor have to be compatible with advanced gastric cancer including gastro-esophageal tumors.
4. Patient has to have adequate bone marrow function, platelet count (≥ 100,000/ µL), hepatic function, neurologic function and blood coagulation parameters.
5. Patient available for oral administration.
6. Estimated life expectancy of patients must be a minimum of 12 months.

7. Patients may receive chemotherapy with FLOT as first-line treatment after surgery.
8. Patient willing and able to comply with scheduled visits, treatment plans, laboratory tests and other study procedures.
9. Patients or a concerned guardian must sign an informed consent indicating that they are aware of the investigation of the study in keeping with the policy of the hospital.
10. Patient must follow authorization permitting release of personal health information.

3.1.3. Exclusion criteria

1. Patients under the age of 20 years.
2. Patients with histopathological diagnosis of any malignant gastric neoplasm other than gastric carcinoma.
3. Patients of gastric carcinoma with distant metastases (lung, liver, brain, etc) or in palliative care.
4. Patients with GI obstruction or other diseases that could provoke nausea and vomiting.
5. Patients who are previously diagnosed and have received prior radiotherapy, chemotherapy and had undergone complete gastrectomy.
6. Pregnant or breast-feeding women and women who is at risk of becoming pregnant during the study period.
7. Patients with acute hepatitis, active infection (bacterial or viral, need parenteral antibiotic treatment), uncontrolled diabetes, serious non-healing wound, bleeding disorder, coagulopathy, and clinically significant proteinuria.
8. Patients with history of or having clinically significant cardiovascular complications which include: myocardial infarction/unstable angina, cardiac arrhythmia and/or uncontrolled hypertension.
9. Patients with clinically significant autoimmune disease uncontrolled with treatment.
10. Patients who have known allergy or severe side effect on study drugs.
11. Those who were not willing to take part in the study.

3.1.4. Ethics and informed consent

The Institutional Ethical Committee of the CNCI approved the study in accordance with the declaration of Helsinki [IEC Ref. CNCI-IEC-DL-2020-6]. The consent form (English, Hindi and Bengali) was signed or thumb impression was taken by the participants. The clinical, demographic and histopathological data were obtained from the patients file in accordance to the given information, their radiological reports and pathological analysis (ANNEXURE I, II and III).

3.1.5. Sample size

Sample size of this study is 78 with power 89% which has been calculated with the help of Epi Info™ (version 2.7.2.2) EPI INFO. As per Wang et. al. (2019) their study found that 59% of the patients needed first-line chemotherapy with FLOT regimen (i.e., p=0.59). Therefore, the number of patients required for this study is 78.03 ~ 78 with the power of 89%.

3.1.6. Evaluation of clinical response

Response to chemotherapy was evaluated after the findings of radiological examination of the stomach using CECT (Contrast-enhanced computed tomography) scan, thorax imaging and PET-CT scan following the completion of the treatment based on Response Evaluation Criteria in Solid Tumors, version 1.1(RECIST, v1.1). The RECIST criteria v1.1 is summarized in **Table 3** (Sando et al., 2023; Eisenhauer et al., 2009). Furthermore, the ECOG performance status of the patients were scored as 0 (asymptomatic, fully active); 1 (completely ambulatory, restricted to physically strenuous activities); 3 (50% confined to bed and capable of limited self-care); 4 (bedbound, completely disabled); and 5 (dead) (Oken et al., 1982).

Table 3: RECIST v1.1. treatment response categorization

Response group	Response of target lesion			Type
	Target lesion	**Non-target**	**New-lesion**	
Complete response (CR)	Disappearance of all target lesions; lymph node axis <10mm	Disappearance of all no non-target lesions; Normalization of tumor marker level	No or partial response	Objective response rate (ORR)
Partial response (PR)	At least a 30% decrease in the sum of diameters of target lesions (SLD) from baseline	No progression	No	
Stable disease (SD)	Neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progression	Persistence of 1 or 2 non-target lesions or tumors	No	Non-responders
Progressive disease (PD)	At least a 20% increase in the sum of diameters of target lesions	Unequivocal progression of lesion size	Yes, appearance new metastatic lesion	

3.1.7. Clinical endpoints

Progression free survival (PFS) is the time from the date of diagnosis until first evidence of disease progression or death (Shitara et al., 2013). Overall survival (OS) is the time interval from the time of diagnosis to death. Patients lost to follow up in last 6 months and could not be contacted were considered as censored, an event for OS analysis (Prinja et al., 2010). Hazard ratios (HR) report the difference of time-to-event endpoints (OS, and PFS) analyses. It compares the risk of an event between control and treatment groups. HR = 1: no difference, HR < 1: lower risk, HR > 1: higher risk of the event compared to the control group (Goel et al., 2010).

3.2. *In-vitro* analysis

3.2.1. Cell Culture

Gastric adenocarcinoma cell line, AGS and, normal kidney cell line HEK293 was obtained from the National Centre for Cell Science (NCCS) Pune. AGS are adherent, drug sensitive tumorigenic cells cultured in Ham's F-12K while HEK293 cells were cultured in DMEM (w/1mM sodium pyruvate) media. Both the media was supplemented with 10% FBS and 1% penicillin/streptomycin. The cells were incubated in a humidifier atmosphere with 5% CO_2 at 37°C. The confluency of flask maintained at 70-80%, for subculturing and cryopreservation. Cells were gently rinsed with 1X PBS. Cells were treated with Trypsin-EDTA solution for detachment and pellet down by centrifugation at 1000 rpm for 5 minutes, resuspended and seeded in a new plates\flasks containing new media. Media was changed when required.

3.2.2. Drug preparation and treatment

A concentrated stock solution is prepared first for 5-fluouracil, oxaliplatin and docetaxel, typically 100 mM/ml. The desired working concentration, using a compatible solvent like DMSO or sterile water or media. The solution is filter-sterilized, aliquoted and stored at 4°C or -20°C. Before each use, the stock is diluted to the appropriate working concentrations (6.25, 12.5, 25, 50 and 100 nM) with pre-warmed cell culture medium and administered for further molecular analyses.

3.2.3. Development of drug-resistant cell line

Drug resistant AGS cells were developed by step-wise increases in drug concentrations of 5FU, oxaliplatin and docetaxel up to critical IC_{50} value. Generally, cells tolerate lower drug doses but reduce cell growth became prominent during different stages of drug-resistant cell line development, an apparent nature of drug-resistant cell (Coley et al., 2004).

Initially, AGS cell line was cultured and MTT was performed to identify the starting dose for commencing the treatment at 10–20% of the chronic IC_{50} dose.

Next, cells were seeded into T25-flask approximately around 20% confluence, followed by drug treatment after 24 hours. Next, drug contained media were added to the flasks readied for drug resistance. Parallelly, a drug free flask was set up for each passage. The drug treated cells then sub-cultured at around 50-70% confluency. The cells were grown in drug free medium for at least couple of passage to recover when became intolerant to drug treatment. During each passage the cells were cryopreserved and revived if the cells of the same passage unhealthy or died. The increase drug concentration was allowed till the dose where cells became tolerant with stable viability and 70% cell number were achieved. That particular drug was selected as the maintenance dose to maintain resistance phenotype of the cells.

Next, IC_{50} values of the AGS and AGS/5FUR, AGS/OxaR and AGS/DoceR were obtained by administrating respective drugs of concentration (0, 6.25, 12.5, 25, 50 and 100 nM/mL). The resistance index (RI) was calculated as the ratio between the IC_{50} value of AGS resistant cells and that of AGS cells to measure the degree of resistance or fold increase of drug resistance in the cells as compared to sensitive cells.

3.2.4. Study of cell morphology alteration

The drug sensitive and drug resistant AGS cells were washed and any changes in cell morphology was examined under a phase contrast inverted microscope at 20x, 40x and 100x and photographed with scale.

3.2.5. MTT assay

The cytotoxic activity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay. The cells were suspended in Corning® 96-well tissue culture plates at a density of 1×10^4 cells /well for 24 hours. Cells were treated with different concentrations of 5-fluorouracil, oxaliplatin, and docetaxel (6.25, 12.5, 25, 50, and 100 nM) for 24 hours at 37°C in a CO_2 incubator. Next, 25 µl of MTT solution (5 mg/ml in PBS) was administrated to each well and incubated for 3 hours, formazan was dissolved by dimethylformamide (DMSO). Finally, the ODs at 570 nm were

measured using a microplate multi-scanner auto-reader (Elisa reader). The experiment was conducted in three independent times in triplicates. The IC_{50} value was estimated from the dose response curve for each concentration using GraphPad prism 8.

3.2.6. Rhodamine 123 accumulation assay using flowcytometry

Rhodamine accumulation was quantified in the blood samples of patients as well as in AGS cells. Rh123 is the substrate of P-gP therefore its accumulation eventually indicates the P-gP activity. For blood sample, first RBCs were lysed, remaining blood components were washed with flowcytometry buffer and incubated with 0.5 µM Rhodamine 123. Subsequently, AGS and resistant AGS cells (AGS/5FUR, AGS/OxaR and AGS/DoceR) were cultured at a density of 1×10^4 for 24 hours in the absence or presence of verapamil, a P-gP inhibitor. Next, cells were harvested and resuspended in culture medium followed by rhodamine123 staining in the dark at 37 °C for 30 minutes. Intracellular Rh123 concentration was determined using flow cytometry (BD, FACS Calibur, LSR fortessa X-20 cell analyzer) under 488 nm excitation and 560 nm emission. (Jouan et al., 2016).

3.2.7. siRNA transfection assay

The reagents used for the transfection include RNAi Max, media with 10% serum without antibiotics, optimum media solution with 2% serum, 100 µM stock of control/scrambled siRNA, and 10uM stock of Sorcin siRNA targeted against SRI gene.

Table 4. Calculation of siRNA preparation for 2ml in 6 well plate

Control/scrambled (25-50nm)	1ul control siRNA in 250 ul optimum solution
Sorcin siRNA	
Dose 5 nM	1ul siRNA in 250ul optimum solution
Dose 10 nM	2ul siRNA in 250ul optimum solution
Dose 20 nM	4ul siRNA in 250ul optimum solution
Dose 40 nM	8ul siRNA in 250ul optimum solution

a) Transfer reagent preparation (RNAi Max)

For each 1.5 ml tube add 5 µl Lipofectamine-2000 of and 250 µl optimum solution and for 6 well plate 30 µl Lipofectamine-2000 and 1500 µl optimum solution is required. Mix by gentle pipetting and incubate 20 minutes at room temperature.

b) Preparation of siRNA complex

Start adding with 250 µl of prepared transfection reagent (RNAi Max) to each tube of Sorcin and control siRNA dose. Mix and incubate for 20 minutes in room temperature. Avoid pipetting or tapping as it may break the siRNA complex.

c) siRNA treatment

The siRNA was transfected dropwise into $1x10^4$ AGS and AGS resistant cells (AGS, AGS/5FUR, AGS/OxaR, and AGS/DoceR) after 20 minutes of incubation. Mix 30 times in a to and fro movement. Cells were grown in 6-well plates and transfection of 20 nmol/mL siRNA in AGS, 30nmol/mL siRNA in resistant cells for 5 hours and were processed further.

3.3. Molecular analysis

3.3.1. Hematoxylin and eosin (H&E) analysis

Histological assessment was performed using H&E staining on 5-µm formalin fixed paraffin embedded tissue sections. The slides were visualized through ZEN Blue imaging software. (Chanjuan et al., 2017).

Well differentiated tissues demonstrated presence of gastric glands arranged in tubular structures cells with well-defined nuclei and borders; Moderately differentiated tissues are irregular glandular structures of variable size and shape with large nuclei; and the poorly differentiated tissues showed large nuclei, mostly signet ring shaped, poorly defined borders and scattered cells. Further, GC subtypes were identified and considered. Tubular adenocarcinoma cells form irregular tubular structures surrounding the neoplastic glandular architecture. The tubular cells exhibit enlarged nucleus, with prominent nucleoli

and infrequent mitotic figures (Soda et al., 2022). The gastric SRCC cells exhibit diffusely infiltrative growth pattern, disrupting the normal glandular architecture. The cytoplasmic morphometry presented a prominent single large cytoplasmic vacuole filled with mucin, displaces the nucleus to the peripheral end of the cell, giving it a typical signet ring like appearance (Zhang et al., 2022).

3.3.2. Immunohistochemistry (IHC)

Five mg of tissue specimen were collected and processed to perform IHC for Sorcin, P-gP, and p-ERK1/2 antibodies. Four-µm sections were prepared from paraffin embedded blocks, dewaxed in xylene, dehydrated by graded alcohol and rinsed in water. After antigen retrieval, the sections were blocked by peroxidase solution and immunostained with; Sorcin, P-gP and ERK1/2 primary antibodies diluted at 1:500. The Peroxidase activity was examined by the chromogenic reaction using diaminobenzidine, counterstained with hematoxylin provided with the Kit, and visualized using the Carl Zeiss microscopy (Carl Zeiss GmbH) and Zen (blue edition) software slide imaging microscope.

3.3.3. IHC scoring

For each case, total of 1000 cells per field was counted at 40x and 100x later scored according to the intensity of nuclear or cytoplasmic staining or extend of positively stained cells.

Staining intensity of Sorcin was scored as 1–3 points % positive cell score. The low expression was considered for weak intensity (Score 1) and <1 % positive cell (Score 0), Intermediate expression for moderate intensity (Score 2) and 1-20 % positive cell (Score 1), High expression for strong intensity (Score 3) and <20 % positive cell (Score 2-4) (Feng et al., 2008).

The staining intensity of ERK 1/2 and P-gP were assessed using the following scoring system: 0 represent no chromogenic signal; 1+ as weak staining; 2+ as focal strong or partial intermediate target cells staining; 3+ for >50% cells with intermediate or strong staining; and 4+ as diffuse strong staining (Tuo et al., 2017).

3.3.4. Qualitative and quantitative polymerase chain reaction (PCR)

a) RNA isolation and quantification

Tissue samples (500 mg – 1 gm) were collected in 1X PBS, and chopped finely. Subsequently, the AGS cells were seeded and grown to exponential growth phase. The cells were treated with desired concentration of drugs (0, 6.25, 12.5, 25, 50 and 100 nM) of 5FU, oxaliplatin and docetaxel. After 24h, cells were washed with ice cold 1X PBS, trypsinized and readied for RNA isolation.

Total RNA from tissues and cells were isolated using the TRIzol (Thermo fisher) reagent. The treated sample was incubated later at 4ºC for overnight to permit complete dissociation of the neucleo-protein complex (Till Transparent). Next, 500 µl of Chloroform was added and shaken vigorously for 1-2 minutes using vortex and incubate for 3-5 minutes in room temperature and repeat twice. Centrifuged the samples at 10,000 g for 15 minutes at 4ºC. Aqueous phase was removed and collect in a new tube. Next, equal amount of isopropanol was added and mixed at room temperature for 5-10 minutes or at -20ºC for 2-3 hours for better yield. Sample was centrifuged at 10,000 g for 10 mins at 4ºC, RNA pellet was obtained as pellet. 1 ml of chilled 75% ethanol was mixed in the sample and invert gently then incubate at -20ºC for 5 minutes. Centrifugation was done at 10,000 g for 15 minutes at 4ºC. Supernatant was discarded and allowed for air dry, later dissolved in DEPC treated water and kept in hot air oven at 65ºC for 2 minutes.

Purity: RNA samples were quantified using nanodrop (Nabi, genetix). The purity of samples was assessed through 260/280 and 260/230 nm absorbance ratios. 260/280 and 260/230 ratios should be around 2 µg for RNA.

b) cDNA preparation

cDNA was prepared using 1µg of mRNA with the one Script cDNA Synthesis Kit (Abm). For first strand synthesis incubate the first mixture at 65ºC for 5 minutes in thermal cycler machine and 1-2 minutes in ice. For second strand synthesis, add the transcript reaction mixtures, incubate mixture 2 at 42ºC for 1 hour in thermal cycler machine. Stop the reaction by heating it at 85ºC for 5

minutes and the newly synthesized the first strand of cDNA is ready for downstream application.

Table 5: Preparation of reaction mixture of 20 µl for cDNA stock

Ingredient	Volume (µl)
RNA Sample	1µg
10mM Oligo dT	2 µl
10mM dNTPs mix	1 µl
TIScript II reaction mix (10X)	2 µl
TIScript II reaction mix (2X)	10 µl
Nuclease free water for volume makeup	9 µl

c) Primer designing

The primer was designed using web tools like Primer3, NCBI Primer-BLAST, UCSC, and the IDT DNA's Oligo Analyzer to identify target sequence. Primer 3 was used to specify primer's melting temperature (Tm), length (usually 18-25 nucleotides), size (typically 100-500 bp), and GC content (40-60%), ensuring that the selected primers are optimal for PCR reactions. IDT DNA's Oligo Analyzer used for calculating the melting temperature, GC content, and the identification of possible secondary structures like hairpins or dimers that could impair PCR efficiency. The UCSC In Silico PCR tool considers the primer specificity, predict amplification of the desired target without binding to unintended sites by reducing the need for empirical trial and error.

Table 6: Primer information

mRNAs	**Primer sequence (3'-5')**		**Annealing Temperature**	**Size (bp)**
Sorcin (SRI)	Forward	GAACTCTGGGCTGTACTGAAT	53 °C	90
	Reverse	CTGCAATTCTTGTGGGTCTACT		
MDR1 (P-gP)	Forward	TGCTCCACTCAGCCAACAAA	56 °C	282
	Reverse	TGGCTAATGAGCTGCGGTTT		
ERK1	Forward	TGTGGCCTTACAAAGGGGGA	55 °C	227
	Reverse	AAGGGTTCAGGGCCAGTAGA		
ERK2	Forward	GCACAATACAGGCCAGAGGT	55° C	227
	Reverse	GCCCACAGGTCTAACAGCAT		
18S	Forward	CTCAACACGGGAAACCTCAC	56° C	90
	Reverse	CGCTCCACCAACTAAGAACG		
GAPDH	Forward	GACAGTCAGCCGCATCTTCT	55° C	221
	Reverse	GCGCCCAATACGACCAAATC		

Table 7: Preparation of PCR reaction mixture of 25 µl

Reagents	Amount
10X PCR buffer	2.5 µl
10Mm dNTPs	1.0 µl
Forward primer	0.25 µl
Reverse primer	0.25 µl
Taq polymerase	0.25 µl
Templete cDNA	50-100ng
Nuclease free water	For volume makeup till 25 ul

Table 8: Thermal cycler machine for set up at the following thermal profile

Process	Temperature	Time	Cycle
Denaturation	94ºC	30 seconds	1
Annealing	55-60ºC	30-45 seconds	35
Extension	72ºC	30 seconds	
Final extension	72ºC	7 minutes	1

The amplified products were subjected to semi-quantitative reverse transcription PCR and real time PCR. The reverse transcriptase PCR was visualized through 1% agarose gel electrophoresis in Chemi gel doc (Bio-Rad) and fold change was estimated. All quantifications were normalized to the level of 18S or GAPDH transcripts, as input control.

d) Real time-PCR

SYBER green-based qRT-PCR was performed according to manufacturer's protocol in the Roche thermocycler using mRNA primers for Sorcin (SRI), MDR1, ERK1 and ERK2 and the results were analysed in Light cycler 96 (v. 1.1.0.1320) software.

3.3.5. Protein isolation

a) Composition of RIPA lysis buffer

The lysate was prepared using RIPA lysis buffer. The 1X RIPA lysis buffer, mix 1X protein cocktail inhibitor (PIC), 0.1 mM PMSF and 0.5 mM NaF to the buffer to make complete lysis buffer.

b) Protein isolation from tissues

Tissue sample was finely chopped on ice, and transferred to round-bottomed microcentrifuge tubes, followed by immediate snap-freezing in liquid nitrogen. For each 5 mg of tissue, 300 µL of ice-cold lysis buffer was added. The tissue was then thoroughly homogenized using an electric homogenizer, with an additional 300-600 µL of lysis buffer added during the process. Post-homogenization, samples were sonicated on ice for 1.5 minutes, followed by centrifugation at 16,000 xg for 20 minutes at 4°C. The supernatant was collected in fresh tubes and stored at -80°C.

c) Protein isolation from cells

AGS, resistant, and siRNA transfected cells were either scraped or trypsinized. Whole cell lysate was prepared by adding cocktail of RIPA lysis buffer, phosphatase inhibitors, PMSF, NaF and protease inhibitor and the mixture was kept on ice for 60 minutes with agitation in between. Samples were then centrifuged at 14,000 rpm for 20 minutes at 4°C, and the supernatant was collected. The tubes were transferred to -80°C deep freezer for storage until molecular analysis were performed. Protein concentration was determined using Bradford reagent, and absorbance was measured at 595 nm using an ELISA plate reader.

3.3.6. Sample preparation

Around 50-80 µg protein lysate was mixed with Laemmli's buffer containing ß-mercaptoethanol in a 1:1 ratio. The mixture was then dry heated at 100°C for 5 minutes using a PCR machine.

3.3.7. Western Blot

The prepared samples (60-80 µg) were separated by 6-12% denaturing SDS-PAGE gel electrophoresis at constant voltage of 60-90 volt. Proteins were then transferred onto a PVDF membrane using the semi-dry transfer method at 15 V for 20-40 minutes, depending on the molecular weight. The membrane was blocked with 5% blocking buffer (BSA or non-fatty milk) for 45-60 minutes at

room temperature, followed by three washes with 1X TBST buffer, 5 minutes each. The blots were incubated overnight at 4°C with primary antibodies against Sorcin (1:1000), ERK1 (1:2000), ERK2 (1:1000), P-gP (1:1000), and β-actin (1:5000). The next day, blots were incubated with HRP-conjugated secondary antibodies (mouse IgG, 1:5000; rabbit IgG, 1:4000) for 1-2 hours at room temperature. Subsequently, blots were washed with 1X wash buffer and visualised by Luminol detection kit via ChemiDoc imaging system (Bio-Rad).

3.3.7. Co-immunoprecipitation (Co-IP)

Cells were lysed with RIPA lysis buffer for 60 minutes on ice then centrifuge at 18,000 xg for 30 min at 4°C. For immunoprecipitation, 500 µg of each lysate was incubated with 2 µg of anti-Sorcin or ERK antibodies on a rotator overnight at 4°C. Subsequently, 100 µl of Protein A agarose bead slurry was added to each sample and incubated with gentle rotation at 4°C for 3 hours. The samples were then centrifuged at 2,500 rpm for 5 minutes at 4°C. The beads were then washed three times with PBS, suspended in 5X concentration. SDS-PAGE loading buffer was added and boiled for 5 min at 100°C. The released proteins (IP) were separated by SDS-PAGE and detected in immunoblot using ChemiDoc (BioRad).

3.4. Statistical analysis

All the clinical and molecular data were statistically analysed using SPSS v16 (IBM Corps., Chicago, IL, USA) and GraphPad Prism 8.0 (Graph Pad Software, Inc.) software. The statistical tests used are explained as follows:

1. Descriptive statistics of sociodemographic, clinicopathological, and treatment outcome to calculate the frequencies of variables, distribution and percentages.
2. Survival outcomes were assessed for the entire study cohort. Kaplan-Meier survival curves were produced and mean OS was estimated, months [95% confidence interval (CI)]. Log rank (mantle-cox) test evaluated the differences in GC subtypes, TA and SRCC, and two different treatment groups- ‘upfront surgery’ and ‘NACT’, in relation to Sorcin expression.

3. Hazard ratio (HR) of presenting GC symptoms and clinicopathological features across different GC subtypes was assessed using Cox regression analyses, HR>1[95% CI; $p<0.05$] to analyze the relative risk associated with various symptoms and clinicopathological characteristics in different GC subtypes.
4. The possible correlations between GC grades and different categorical clinicopathological variables and H-scores of the genes and clinicopathological factors were analyzed using cross tabulation. Pearson's $\chi 2$ test statistically observed the significance ($p=0.05$) of the relationships.
5. Gene expression levels were measured using CT values and normalized to the housekeeping gene GAPDH and 18s. The ΔCT method was used for normalization, and mean ± standard error was calculated. The relative fold change in mRNA expression was estimated using the 2^(-ΔΔCT) method. To evaluate differences in fold change between GC, different GC subtypes, and treatment groups, unpaired t-tests were used for comparisons between two groups.
6. Protein expression was quantified using densitometry in Image J imaging software and normalized to the housekeeping protein beta-actin. Unpaired t-tests were used to compare fold changes between two groups, while one-way ANOVA was performed across three or more groups.
7. The observed differences considered significant at $p \leq 0.05$.
8. Positive correlation was evaluated using Pearson's correlation coefficient to assess the relationship between the expression levels of two genes.
9. Nonlinear regression analysis was performed on the transformed and normalized MTT values using nonlinear curve fitting. The analysis evaluated the calculated log IC_{50} values, IC_{50} values, and Hill slope values. Additionally, the goodness of fit was assessed using R-squared values (≥ 1). An unpaired t-test was further employed to assess any significant differences between the groups.

3.5. Chemicals

A list of chemicals used in the study are attached in ANNEXURE IV.

CHAPTER 4
RESULTS

4.1. Clinical assessment of gastric cancer (GC) patients

4.1.1. Socio-demography

Among 78 patients diagnosed with gastric adenocarcinoma, the majority were in the 41-60 age group, with a median age of 52 ± 11.35 years. Of these patients, 54 were male (69.2%), rural resident 58 (74.4%), Hindus 61(78.2%) and majority of them belonged to lower socioeconomic classes, with 41labourers (52.5%) and 42 of them (53.8%) having an income range of 2,000-10,000 rupees. The cohort predominantly followed a mixed diet, 69 individuals (88.5%) consisting of highly salted food, meat, smoked foods contaminated oily junk and high fat foods. For addictions, combination of smoking, alcohol and tobacco was testified by 22 patients (28.2 %) having past history of peptic disorder (60.3%) (**Table 9**).

Table 9. Socio-demographic distribution of GC patients

Characteristics (n=78)		**Frequency (%)**
Age (years)	19-40	11(14.1)
	41-60	**47(60.3)**
	>60	20(25.6)
Gender	**Male**	**54(69.2)**
	Female	24(30.8)
Habitation	**Rural**	**58(74.4)**
	Urban	20(25.6)
Religion	**Hindu**	**61(78.2)**
	Muslim	17(21.8)
Language	**Bengali**	**71(91.0)**
	Hindi	7(9.0)
Occupation	**Labor**	**41 (52.5)**
	Government or Private farm employee	22 (28.2)
	Unemployed	15 (19.2)
Income (Rs/-)	<2000	1(1.3)
	2001-10000	**42(53.8)**
	10001-20000	29(37.2)
	<20000	6(7.7)
Marital status	Un-married	14(17.9)
	Married	**47(60.2)**
	Widowed	17(21.7)

Diet	**Mixed diet**	**69(88.5)**
	Vegetarian	9(11.5)
Addiction history	Nil	25(32.1)
	Smoking	**21(26.9)**
	Alcohol	6(7.7)
	Tobacco chewing	4(5.1)
	Smoking, alcohol and tobacco	**22(28.2)**
Family history	No history	70(89.7)
	Gastric ulcer	3(3.8)
	Gastritis	3(3.8)
	Malignancy	2(2.6)
Past history of the patient	Nil	30(38.5)
	Peptic disorder	**47(60.3)**
	Carcinoma larynx, chronic gastritis	1(1.3)
n= total number of individuals; %=percentage		

4.1.2. Initial symptoms

At diagnosis, 72 patients experienced abdominal pain (97.4%) and 69 of them had progressive weight loss (89.7%), however dysphagia was less commonly seen in only 12 of the patients (8.9%). The other important presenting feature of advanced GC is anaemia experienced by 48 patients (67.9%) and epigastric lump in 44 individuals (55.1%) (**Figure 10**).

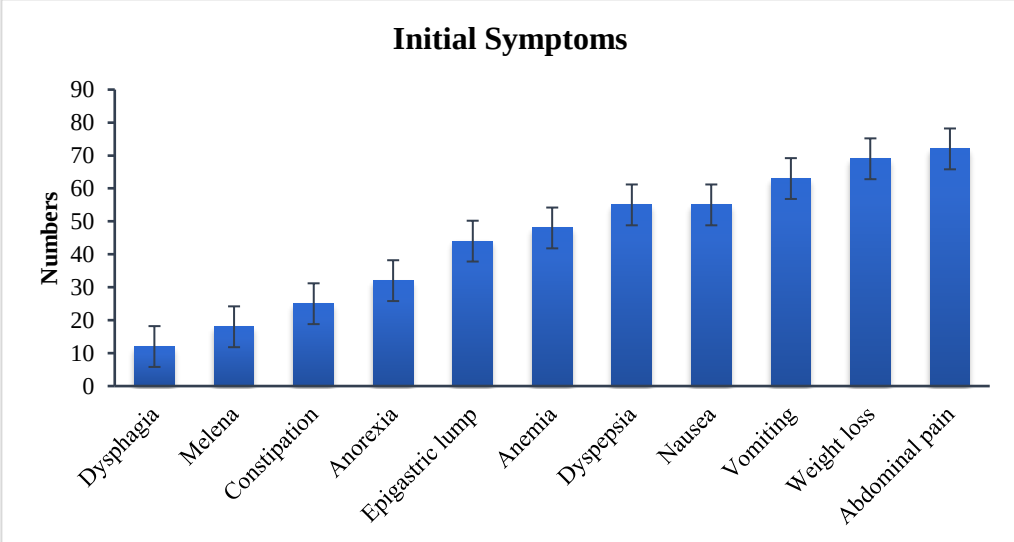


Figure 10: Incidence of initial symptoms experienced by GC patients.

4.1.3. Clinicopathological features

The most common site of tumour growth is the antrum (70.5%), with a tumour size of ≥5 cm observed in 56.4% of cases. Additionally, wall thickening was the

most prevalent feature, occurring in 60.3% of individuals. The ECOG performance status indicated that the majority of patients (47, 60.3%) were bedridden 50% of the time but remained capable of self-care (**Table 10**).

Table 10. Clinicopathological features of GC patients

Characteristics (n=78)		**Frequency (%)**
Tumor type	**Adenocarcinoma**	**77 (98.7)**
	Gastrointestinal stromal tumor (GIST)	1 (1.3)
Primary tumor site	**Antrum**	**55 (70.5)**
	Pylorus	7 (9.0)
	Body	8 (10.3)
	Lesser curvature	2 (2.6)
	Fundus	2 (2.6)
	Gastroesophageal (GE) junction	4 (5.1)
Tumor size at diagnosis	≤5 cm	34 (43.6)
	≥5 cm	**44 (56.4)**
Radiological findings	Ulceration	12 (15.4)
	Growth	16 (20.5)
	Wall thickening	**47 (60.3)**
	Wall thickening and ulceration	2 (2.6)
	Patchy erythema	1 (1.3)
Eastern cooperative oncology group (ECOG) performance status	0	1 (1.3)
	1	19 (24.4)
	2	**47 (60.3)**
	3	7 (9.0)
	4	4 (5.1)
n= total number of individuals; %=percentage		

According to the Borrmann classification, the majority of patients (55.1%) had Type II (fungating) carcinoma. The Lauren classification revealed that 45 patients (57.7%) exhibited the intestinal type of GC, while 33 (42.3%) had the diffuse type.

Based on the WHO classification, the majority of patients (47.4%) had TA, followed by SRCC (41.0%), with poorly differentiated histology observed in 57.7% of cases. Additionally, 57 patients (73.3%) had T3 tumour extent, while 27 (33.3%) showed N2 lymph node involvement.

Overall, the largest proportion of patients were at stage IIIA (34.6%), followed by IIIB (24.4%). Lymphovascular invasion (LVI) was present in 75.6% of cases, perineural invasion (PNI) in 29.5%, and serosal involvement (SI) in 19.2% of patients (**Table 11**).

Table 11. Clinical findings of GC patients at diagnosis

Characteristics (n=78)		**Frequency (%)**
Borrmann classification	Type I (polyploid tumor)	1 (1.3)
	Type II (fungating carcinoma)	**43 (55.1)**
	Type III (ulcerated carcinoma)	26 (33.3)
	Type IV (infiltrating carcinoma)	8 (10.3)
Lauren classification	**Intestinal type**	**45 (57.7)**
	Diffuse type	33 (42.3)
WHO classification	**Tubular adenocarcinoma**	**37 (47.4)**
	Mucinous adenocarcinoma	6 (7.7)
	Signet-ring cell carcinoma	**32 (41.0)**
	Poorly cohesive carcinoma	3 (3.8)
Grade	Well differentiated	4(5.1)
	Moderately differentiated	29(37.2)
	Poorly differentiated	**45(57.7)**
pT-stage	T1ab	2 (2.6)
	T2	8 (10.3)
	T3	**57 (73.3)**
	T4a	11 (14.1)
pN-stage	**N0**	**21 (26.9)**
	N1	8 (10.3)
	N2	**26 (33.3)**
	N3ab	**23 (29.5)**
pM-stage	M0	76 (97.4)
	Mx	2 (2.6)
pTNM Stage	IA	2 (2.6)
	IB	4 (5.1)
	IIA	17 (21.8)
	IIB	5 (6.4)
	IIIA	**27 (34.6)**
	IIIB	**19 (24.4)**
	IIIC	4 (5.1)
Lymphovascular invasion (LVI)		**59 (75.6)**
Perineural invasion (PNI)		23 (29.5)
Serosal invasion (SI)		15(19.2)
n= total number of individuals; %=percentage		

4.1.4. Treatment modalities

A total of 49 patients (62.8%) underwent upfront surgery followed by adjuvant chemotherapy (ACT), while 29 (37.2%) received neoadjuvant chemotherapy (NACT) followed by surgery. Distal gastrectomy was performed in 69.2% of cases, most commonly with D2 lymphadenectomy. Additionally, 38.5% of patients received capecitabine combined with oxaliplatin (CAPOX) as ACT, with at least 42.2% completing six cycles. In contrast, 37.2% of NACT recipients were treated with four cycles of the FLOT regimen perioperatively. Notably, the recurrence rate was 11.5%, with local recurrence being more

common than regional recurrence. The survival time for the majority of patients (28, 35.9%) ranged from 7 to 12 months (**Table 12**).

Table 12: Treatment approaches of GC patients

Characteristics (n=78)			**Frequency (%)**
Treatment modalities	**Upfront surgery followed by ACT**		**49 (62.8)**
	Neoadjuvant chemotherapy (NACT)		29 (37.2)
Surgical Approach	Total gastrectomy		5 (6.4)
	Proximal gastrectomy		5(6.4)
	Distal (Subtotal) gastrectomy		**68(87.1)**
Lymphadenectomy	D1+		4(5.1)
	D2& D2+		**74(94.8)**
Chemotherapy regimen	ACT	Capecitabine	15 (19.2)
		CAPOX	**30 (38.5)**
	NACT	FLOT	**29 (37.2)**
	None		4 (5.1)
Chemotherapy cycles	ACT (n=45)	2	10(22.2)
		3	5(11.1)
		4	4(8.9)
		6	**19(42.2)**
		8	3(6.7)
		12	4(8.9)
	NACT (n=29)	4	29 (100)
	Nill		4(5.1)
Recurrence	**Local recurrence**		**9 (11.5)**
	Regional recurrence		**2 (2.5)**
	No recurrence		67 (85.8)
Survival status	**Dead**		**56 (71.7)**
	Censored		22 (28.2)
Survival (months)	0-6		9 (16.1)
	7-12		**25 (44.6)**
	13-19		11 (19.6)
	20-26		7 (12.5)
	27-33		2 (3.6)
	<33		2 (3.6)
n= total number of individuals; %=percentage			

4.1.5. Treatment evaluation of GC patients after chemotherapy

The overall treatment response, including complete responses (CRs) and partial responses (PRs), was observed in 17 (21.8%) patients. Among ACT recipients, 27 (55.1%) were non-responders (NRs), while in the NACT group, 17 (58.6%) were NRs. Moreover, the majority of patients (52, 66.7%) experienced post-chemotherapy complications, with malnutrition being the most prevalent, affecting 48 patients (61.5%) **(Table 13)**.

Table 13: Assessment of treatment response among the NACT patients

Characteristics (n=78)			**Frequency (%)**
Overall treatment response		CRs	17 (21.8)
		PRs	17 (21.8)
		NRs	**44 (56.4)**
Treatment Response	ACT (n=49)	ORR	22 (44.9)
		NRs	**27 (55.1)**
	NACT (n=29)	ORR	12 (41.3)
		NRs	**17 (58.6)**
Post-treatment complications		**Present**	**52 (66.7)**
Post-treatment nutritional status		Good	8 (10.3)
		Average	22 (28.2)
		Malnourishment	**48 (61.5)**
n= total number of individuals; %=percentage; CRs- complete responders, PRs-partial responders, NRs-non-responders, ORR- overall response rate (CRs + PRs).			

4.2. Impact of initial symptoms, clinicopathological features and treatment outcomes in overall survival

Overall, 56 patients (71.7%) were succumbed to death and 22 (28.2%) were censored. The mean OS of the patients was 19.7 months [95% CI: 15.9-23.5] (**Figure 11).**

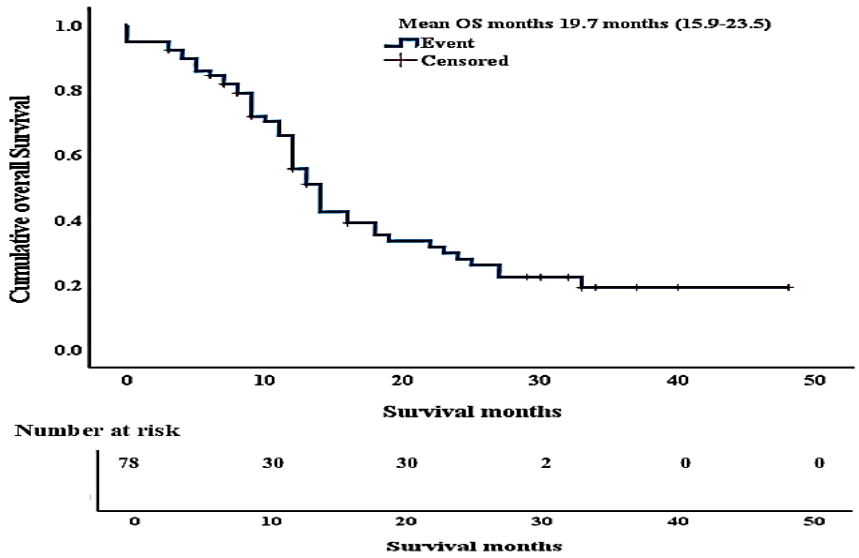


Figure 11: Kaplan-Meier curve of overall survival of entire studied GC population.

Patients who experienced weight loss, had a significantly lower OS, of about 18.3 months (95% CI: 14.2-22.5), (p = 0.038). This group also demonstrated a significantly higher HR of 2.84 (95% CI: 1.01-7.99), (p = 0.047). Additionally, other symptoms, except constipation, such as nausea, vomiting, and melena, were associated with higher hazard ratios (HR >1), however, they did not reach statistical significance **(Figure 12)**.

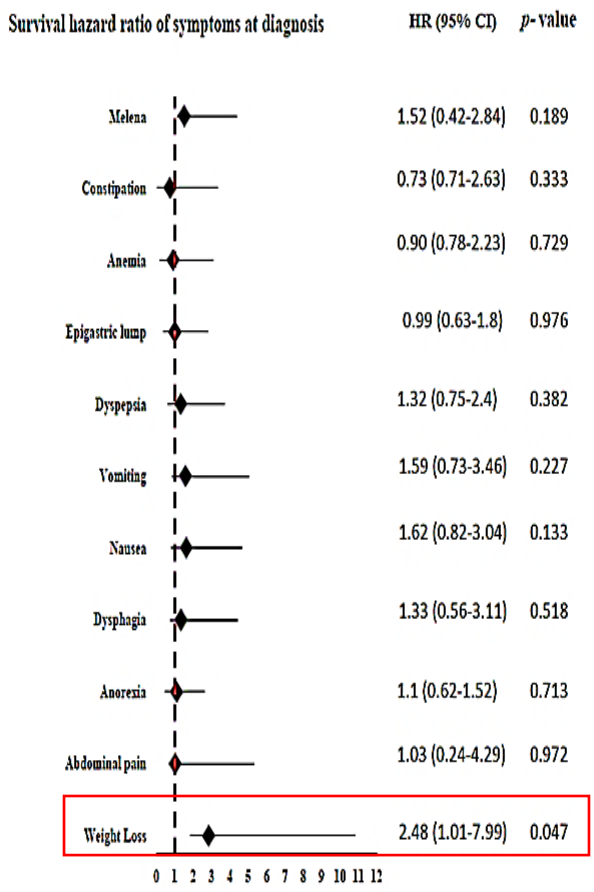


Figure 12: Initial symptoms of GC patients at diagnosis with overall survival and hazard risk.

Furthermore, the OS is significantly lower in age group >60 years, [10.67 months (95% CI: 6.7-14.6), (p<0.001)] with an HR of 2.003 [95% CI: 1.2-2.3] (p = 0.002); Tumors located in the fundus had the lowest OS at 4.50 months [95% CI: 3.52-5.82], but the connotation was not significant (p = 0.054), with an HR of 0.977 [95% CI: 0.83-1.13]. Patients with pTNM stage III had an OS of 16.5 months [95% CI: 12.3-20.7], however the p-value of 0.287 was not significant, with HR was 1.24 [95% CI: 0.97-1.6], (p = 0.082). Moreover, overall tumor size, grade, PNI and SI indicated HR>1, and although the risk is nonsignificant (p>0.05) (**Table 13**).

Table 13: OS and HR with clinicopathological features in GC patients

Characteristics (n=78)		**Kaplan-Meier survival (Univariate)**			**Cox regression (Multivariate)**		
		Mean OS (month)	95% CI	p-value	HR	95% CI	p-value
Age (Years)	19-40	18.05	10.27-26.34	**0.001**	**2.003**	1.2-2.3	**0.002**
	41-60	23.28	18.44-29.17				
	>60	**10.67**	6.78-14.62				
Gender	**Male**	**17.84**	13.49-22.18	0.135	**1.57**	0.84-2.9	0.147
	Female	20.18	15.49-24.87				
Diet	**Mixed diet**	20.39	16.15-24.62	0.557	0.803	0.37-1.71	0.569
	Vegetarian	**15.55**	7.47-23.63				
Primary tumor site	Antrum	17.71	13.76-21.76	**0.054**	0.977	0.83-1.13	0.769
	Pylorus	13.02	6.78-19.27				
	Body	22.12	13.79-30.45				
	Lesser curvature	15.0	9.12-20.88				
	Fundus	**4.50**	3.52-5.82				
	GEJ	22.75	7.01-38.48				
Tumor size	**≤5 cm**	**15.62**	11.8-19.4	0.297	**1.30**	0.43-1.3	0.344
	≥5 cm	22.5	16.5-28.4				
Lauren classification	Intestinal type	19.20	14.12-24.31	0.392	0.791	0.53-1.6	0.406
	Diffuse type	18.07	13.45-23.95				
WHO classification	Tubular adenocarcinoma	20.02	14.54-25.50	0.458	0.883	0.72-1.22	0.351
	Mucinous adenocarcinoma	**9.50**	4.61-14.38				
	Signet-ring cell carcinoma	**16.61**	12.14-21.09				
	Poorly cohesive carcinoma	29.00	13.75-44.24				
Grade	I	19.5	2.2-36.7	0.650	**1.15**	0.72-1.8	0.557

	II	20.0	13.9-26.2				
	III	**17.2**	12.9-21.5				
LVI	Present	19.01	14.18-23.84	0.296	0.723	0.38-1.3	0.288
	Absent	20.26	15.14-25.37				
PNI	Seen	17.5	13.0-22.0	0.585	**1.17**	0.64-2.1	0.545
	Not seen	19.2	14.4-24.0				
SI	Present	24.6	14.9-34.3	0.319	**1.42**	0.69-2.9	0.520
	Absent	**16.4**	13.2-19.6				
pT-stage	T1ab	32.6	25.7-39.6	0.355	**1.32**	0.82-1.8	0.194
	T2	**16.7**	7.3-26.1				
	T3	17.7	13.2-22.3				
	T4a	19.7	10.9-28.4				
pN-stage	N0	23.1	15.9-30.3	0.287	**1.24**	0.97-1.6	0.082
	N1	18.8	10.2-27.4				
	N2	17.4	11.9-22.9				
	N3 (a,b)	**12.6**	7.9-17.3				
pTNM stage	I	29.0	22.0-35.9	0.159	**1.46**	0.93-2.2	0.081
	II	18.1	11.6-24.7				
	III	**16.5**	12.3-20.7				

Furthermore, patients receiving NACT with the FLOT regimen had a significantly lower mean OS of 17.7 months [95% CI: 11.9–23.08], (p = 0.001), with a substantially high HR of 23.5 [95% CI: 6.3–88.21], (p<0.001). Additionally, patients who underwent six cycles of ACT had a mean OS of 22.5 months [95% CI: 6.7–25.2], with both OS and HR showing statistical significance (p<0.001) and an HR of 1.26 [95% CI: 0.28–5.67]. Notably, undernourished NACT patients had a lower mean OS of 16.49 months [95% CI: 12.7–20.6], (p = 0.373), with an elevated HR of 1.32 [95% CI: 0.81–1.73], (p=0.174) (**Table 14**).

Table 14: OS and HR with treatment outcomes in GC patients

Characteristics (n=78)			**Kaplan-Meier survival**			**Cox regression**		
			Mean OS (month)	95% CI	p-value	HR	95% CI	p-value
Treatment modality	Upfront surgery		20.1	15.2-25.1	0.582	**1.16**	0.64-2.00	0.593
	NACT		17.7	11.9-23.04				
Mode of gastrectomy	Total gastrectomy		16.8	6.2-27.3	0.282	**1.00**	0.76-1.33	0.961
	Subtotal gastrectomy		17.3	9.8-24.7				
	Proximal gastrectomy		11.0	3.8-18.1				
	Distal gastrectomy		**19.9**	15.2-24.7				
Chemotherapy regimen	ACT	CAPOX	21.7	17.1-26.5	**0.001**	**1.36**	0.76-2.44	**0.001**
	NACT	**FLOT**	**17.6**	12.2-22.9		**23.5**	**6.3-88.21**	
	None		0.00	0.00-0.00		0.00	0.00-0.00	
Chemotherapy cycles	ACT	Nil	0.00	0.00-0.00	**0.001**	**1.26**	0.28-5.67	**0.001**
		2	20.9	11.1-30.8				
		3	**7.2**	4.7-9.7				
		4	24.6	16.1-33.1				
		6	22.5	16.4-28.6				
		8	**16.2**	6.7-25.2				
		12	**16.00**	9.1-23.3				
Overall treatment response	CRs		19.50	13.7-24.0	0.289	**1.22**	0.87-1.7	0.235
	PRs		24.57	13.3-34.1				
	NRs		**16.09**	11.6-20.2				
Treatment response	ACT	CRs	22.3	16.5-28.1	0.598	**1.54**	0.622-3.8	0.618
		PRs	23.7	12.4-35.01				
		NRs	**17.4**	12.1-22.6				

	NACT	CRs	14.7	7.1-22.3	0.726	0.56	0.05-3.5	0.743
		PRs	20.0	3.9-36.0				
		NRs	**16.5**	9.6-23.4				
Nutritional status after chemotherapy	Good		22.02	15.9-29.6	0.373	**1.32**	0.81-1.83	0.174
	Average		22.17	14.5-29.8				
	Malnourishment		**16.49**	12.7-20.6				
Post treatment complications	Present		18.71	13.8-23.7	0.328	0.97	0.54-1.72	0.917
	absent		19.44	14.8-24.2				

4.3. Histopathological characteristics of GC

Out of 78 adenocarcinoma patients, majority of them underwent D2 lymphadenectomy, and their pathological grades were assessed using H&E staining **(Figure 13)**. Most tissues with advanced grades were characterised with either tubular adenocarcinoma or signet ring cell carcinoma.

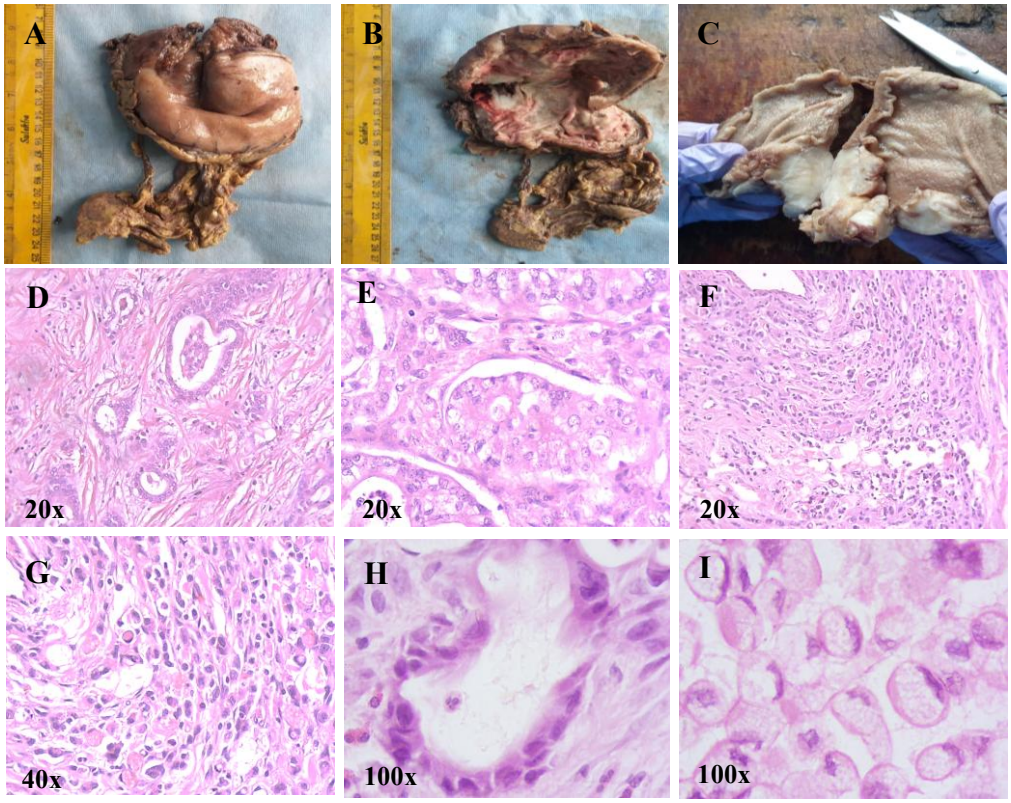


Figure 13: A-C. Total D2 lymphadenectomy of stomach with tumor. The plates showing haematoxylin and eosin staining of **D-** well differentiated; **E-** moderately differentiated; **F-** poorly differentiated (cohesive adenocarcinoma); **G-** poorly differentiated (signet ring cell carcinoma); (20x), **H&I.** gastric TA and SRCC subtype (100x).

4.3.1. Correlation of grades with clinicopathological characteristics

A correlation analysis was conducted to evaluate the impact of tumour grade differentiation on various clinicopathological features. The χ^2-test revealed significant associations between grade differentiation and parameters including age ($p = 0.009$), gender ($p = 0.03$), presence of an epigastric lump ($p = 0.028$), tumour size ≥5 cm ($p<0.001$), and wall thickening ($p = 0.002$). Additionally, higher tumour grades were significantly correlated with Borrmann Type III classification ($p<0.001$), diffuse-type GC ($p<0.001$), and SRCC subtype ($p<0.001$). Moreover, aggressive pathological features such as LVI ($p<0.001$), pT3 tumour extent ($p<0.001$), pN2 and pN3a lymph node invasion ($p<0.001$), and pTNM stage IIIA ($p = 0.001$) showed strong associations with grade differentiation. Notably, postoperative undernourishment ($p = 0.041$) was also significantly linked to tumour grade **(Table 15)**.

Table 15: Correlation of GC grade with clinicopathological features

Variables		**Well differentiated n (%)**	**Moderately differentiated n (%)**	**Poorly differentiated n (%)**	***p*-value**
Age (years)	19-40	0 (0)	1(3.4)	13(28.8)	0.009
	41-60	2(50.0)	**24(82.7)**	**21(46.7)**	
	>60	2(50.0)	4(13.7)	11(24.4)	
Gender	Male	2(100.0)	**24(82.7)**	26(57.8)	0.003
	Female	0(0)	5(17.2)	19(42.2)	
Epigastric lump		1 (2.2)	12(27.3)	**31(70.4)**	0.028
Tumor size	≤5 cm	4(100.0)	18(62.1)	12(26.7)	0.001
	≥5 cm	0(0)	11(37.9)	**33(73.3)**	
Radiological findings	Growth	2(50.0)	7(24.1)	7(15.5)	0.002
	Wall thickening	1(25.0)	18(60.1)	**31(68.9)**	
	Patchy erythema	1(25.0)	0(0)	0(0)	
	Ulceration	0(0)	6(20.6)	7(15.5)	
Bormann classification	Type I	0(0)	1(3.4)	0(0)	0.001
	Type II	4(100.0)	**25(86.2)**	14(31.1)	
	Type III	0(0)	2(6.8)	**24(53.3)**	
	Type IV	0(0)	1(3.4)	7(15.5)	

Lauren classification	**Intestinal type**	4(100.0)	**29(100.0)**	12(44.4)	0.001
	Diffuse type	0(0)	0(0)	33(73.3)	
Lymphovascular invasion		2(3.4)	16(27.1)	**41(69.5)**	0.001
pTNM stage	I	2(50.0)	3(10.3)	1(2.2)	0.001
	II	2(50.0)	13(44.8)	7(15.5)	
	III	0(0)	13(44.8)	**37(82.2)**	
Post treatment nutritional status	Good	2(50.0)	3(10.3)	3(6.6)	0.041
	Average	0(0)	11(37.9)	11(24.4)	
	Malnourished	2(50.0)	15(51.7)	**31(68.9)**	
P is significant at 0.05					

4.4. Expression pattern of Sorcin in GC

4.4.1. Gene expression analysis

Gene expression analysis of Sorcin (SRI) in normal adjacent and GC tissues revealed significant differences in expression levels. The results demonstrated that the mean mRNA level of SRI was markedly higher in GC tissues (3.17-fold) compared to adjacent normal tissues (1.76-fold), with a mean difference (±SEM) of 1.41±0.505 (p=0.0077). Additionally, within different histological subtypes, SRI expression in SRCC showed a 5.46-fold increase compared to TA, which exhibited a 4.038-fold change, with a mean difference of 1.48±0.556 (p = 0.021). These findings highlight the elevated expression of SRI in GC, particularly in SRCC (**Figure 14)**.

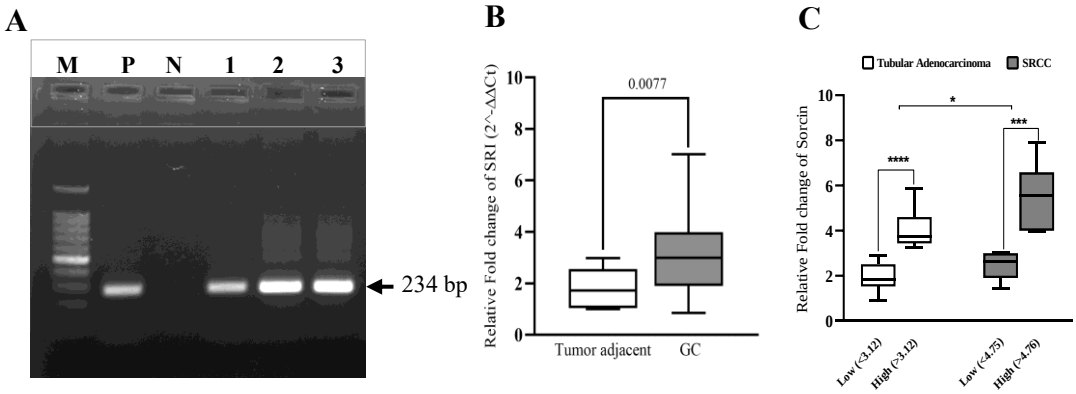


Figure 14: Gene expression of Sorcin (SRI) in GC tissues. A. The 2 % gel image represents the RT-PCR of SRI (M= marker, P= positive control, N= negative control, 1,2 & 3 = GC samples). **B.** Real-time PCR analysis of SRI in tumor adjacent and gastric cancer tissues. **C.** GC subtypes, TA and SRCC presented significant difference in the SRI level. P-values were calculated using unpaired t-test for comparison with significance at *p<0.05, ***p<0.001, ****p<0.0001.

4.4.2. Protein expression analysis

Furthermore, immunostaining of Sorcin was scored compared to negative control. The majority of patients (60.3%) demonstrated strong Sorcin expression (H-score 3). Notably, poorly differentiated adenocarcinoma exhibited significantly higher Sorcin expression (71.1%; p = 0.0001) compared to well- and moderately differentiated GC tissues (24.1%; p = 0.0007; 50%, p = 0.0005, respectively). The intensity of Sorcin expression was markedly higher in both TA and SRCC, as well as in intestinal- and diffuse-type cases (**Figure 15 A-H**).

To further confirm protein expression levels, western blotting was performed, revealing differential Sorcin expression across samples. While some cases exhibited reduced expression (P1= 40% downregulation), a significant proportion displayed higher Sorcin expression in GC tissues (P2-4 = >1.2 fold) compared to adjacent normal tissues with respect to loading control, beta actin (**Figure 15 I**).

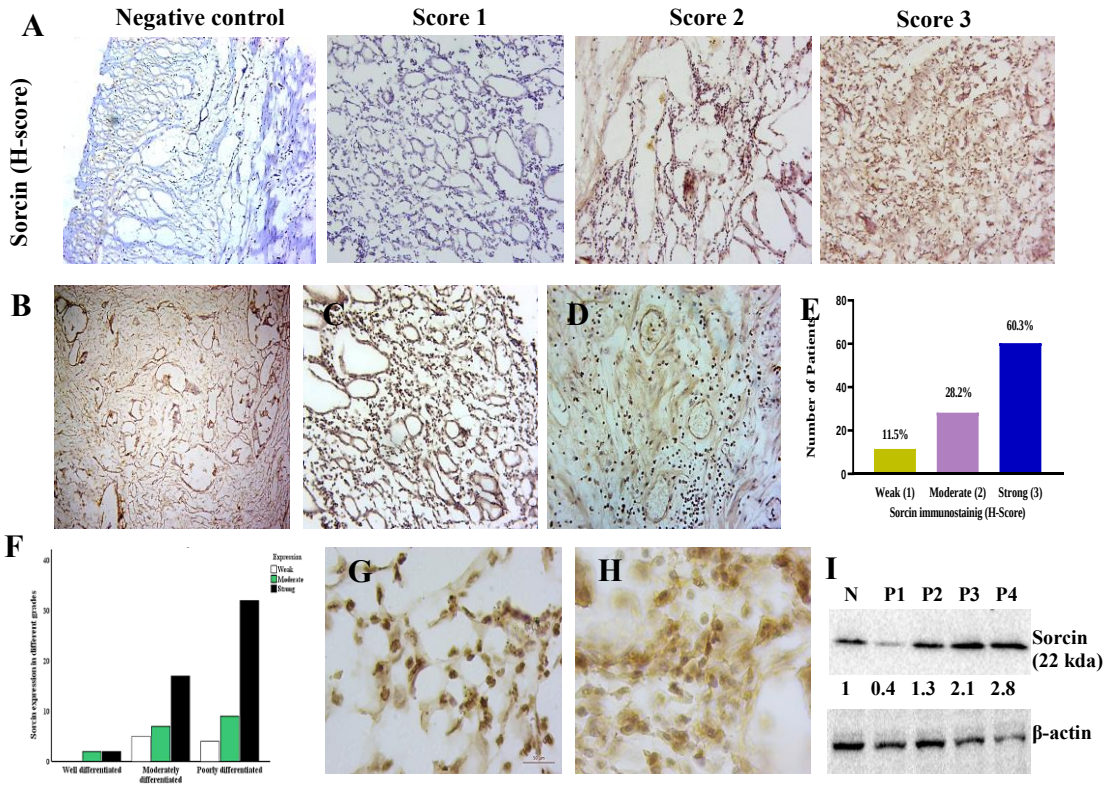


Figure 15: Protein expression of Sorcin in GC tissues. A. Immunohistochemistry images of Sorcin expression representing staining based on H-

score. Sorcin expression in **B.** well; **C.** moderately **D.** poorly differentiated tissue grades (20X). **E.** The graph illustrates the distribution of Sorcin expression with respect to H-score. **F.** Frequency of patients diagnosed with different grades showing Sorcin expression (A-D in 20X). **G&H.** TA and SRCC, (100X). **I.** Western blot representing the Sorcin expression between tumor adjacent (N) and GC patients (P1-4).

4.5. Differential expression of Sorcin in response to neoadjuvant FLOT therapy

The mRNA and protein levels of Sorcin expression were evaluated among patients who underwent neoadjuvant FLOT chemotherapy (NACT, n = 29). The fold change of Sorcin expression was assessed relative to GAPDH in GC patients. A majority of the patients who underwent upfront surgery demonstrated strong Sorcin expression (3.4-fold; p = 0.0001) (**Figure 16 A**). Notably, the mRNA levels of Sorcin were significantly upregulated in NACT recipients compared to upfront surgery cases, with a 2.7-fold increase (p = 0.042). Additionally, Sorcin expression was significantly higher in SRCC than in TA cases (p = 0.0432) (**Figure 16 B**). The results were further validated by reverse transcriptase PCR (**Figure 16 B**). The relative fold change of SRI was notably higher in SRCC (7.5-fold) compared to TA (5.8-fold) (**Figure 16 C**). Furthermore, immunostaining of Sorcin in both TA and SRCC demonstrated a similar trend, with increased expression in response to FLOT chemotherapy (**Figure 16 D-E**). A majority of patients in the cohort had an H-score of 3 for Sorcin immunostaining. Western blot analysis further confirmed that Sorcin expression increased two-fold following NACT (**Figure 16 F**).

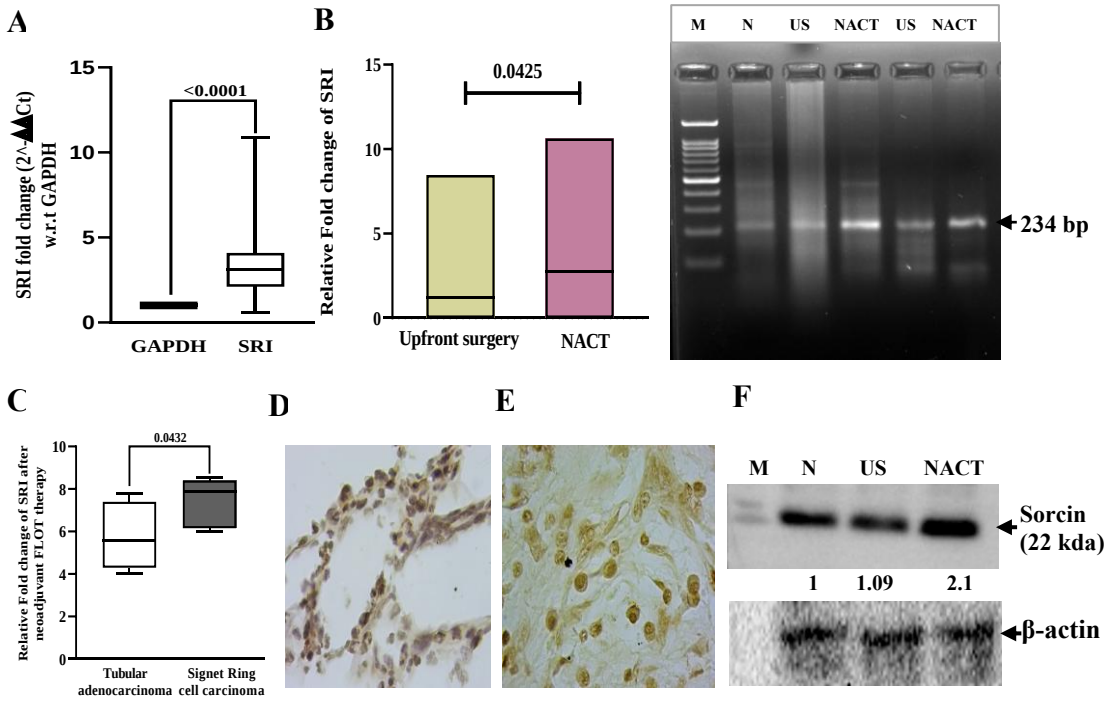


Figure 16: Sorcin level in neoadjuvant FLOT chemotherapy receiving patients. **A**. SRI fold change between GAPDH and SRI. **B.** mRNA level of Sorcin in patients underwent upfront surgery and NACT. The gel representing the SRI level in patients received upfront surgery (US) and NACT. **C.** SRI level in TA and SRCC cases. **D&E.** IHC plates (left) showing Sorcin expression in tissues after neoadjuvant FLOT chemotherapy. **F.** Western blot represents Sorcin expression in patients underwent upfront surgery and neoadjuvant FLOT-chemotherapy.

4.6. Correlation of Sorcin with clinicopathological parameters

Next, the correlation between Sorcin expression and key prognostic clinicopathological features of GC was evaluated. Statistical analysis revealed that Sorcin expression was significantly associated with epigastric lump, Type II Borrmann classification, tumor size >5 cm, presence of LVI, SI, pTNM stage III—specifically pT3 tumor extent and pN2-3 lymph node involvement—as well as post-treatment undernourishment in both TA and SRCC cases ($p < 0.05$) (**Figure 17**).

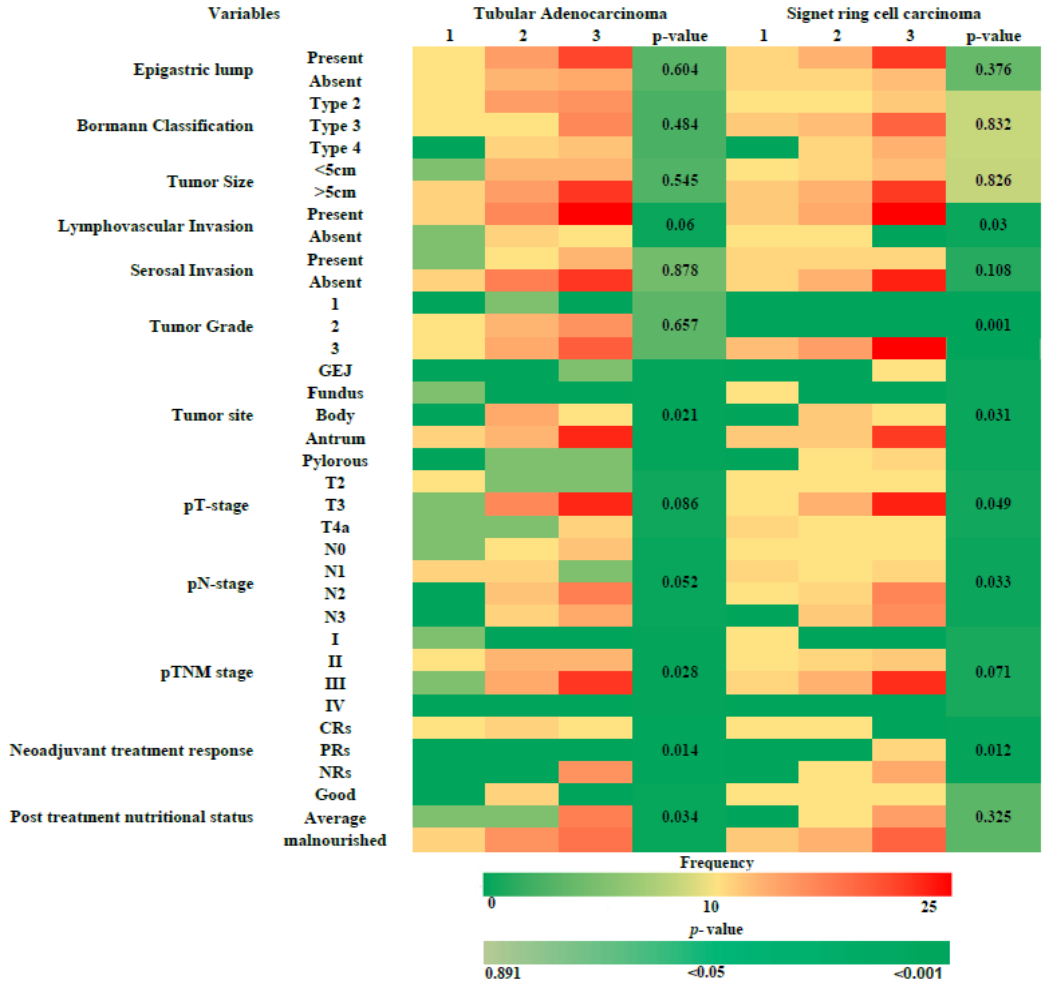


Figure 17: The Heatmap representing relationship between Sorcin expression and clinicopathological factors in TA and SRCC. 1,2,3 represents the weak, moderate and high expression of Sorcin respectively.

4.7. Sorcin correlates P-gP and ERK1/2 expression among GC patients

4.7.1. Gene expression analysis

The drug resistance property of Sorcin was further validated by assessing its association with P-gP/MDR1 and ERK1/2 expression at both the mRNA and protein levels. Real-time PCR analysis confirmed the presence of MDR1, ERK1, and ERK2 mRNAs in TA and SRCC tissues, with mean expression levels of 3.63 ± 1.35, 5.35 ± 0.43, and 4.75 ± 0.72, respectively. Expression values above or below the mean were considered high or low, respectively.

Interestingly, SRCC tissues demonstrated a significant upregulation of MDR1 ($p = 0.009$), ERK1 ($p = 0.0003$), and ERK2 ($p = 0.079$) compared to TA **(Figure**

18 A–C). However, MDR1 expression did not significantly differ between upfront surgery (4.1-fold) and NACT recipients (3.7-fold, p = 0.719) (**Figure 18 D)**. Similarly, ERK1 expression was 3.4-fold in upfront surgery cases and 5.1-fold in NACT cases (mean difference: 1.71 ± 0.9836, p = 0.719), while ERK2 expression was 1.7-fold in upfront surgery cases and 3.8-fold in NACT cases (mean difference: 2.1 ± 0.6636, p = 0.060) **(Figure 18 E–F)**.

Notably, ERK1/2 expression followed a similar trend as Sorcin, unlike P-gP, suggesting that Sorcin may play a distinct role in drug resistance compared to P-gP and ERK1/2. Further RT-PCR analysis revealed elevated expression of Sorcin (>2-fold), P-gP (4.2-fold), and ERK1/2 (2.5-fold) among NRs who underwent NACT. More precisely, Sorcin expression in CRs exhibited a 50% downregulation, a trend not observed in P-gP and ERK1/2 **(Figure 18 G)**.

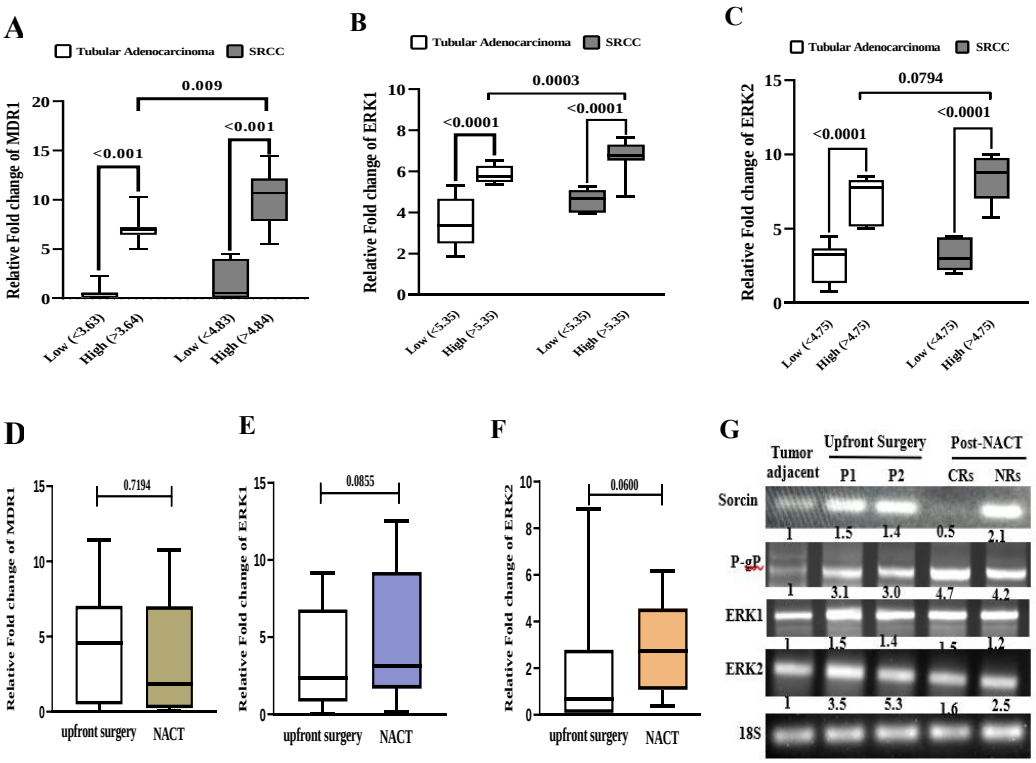


Figure 18: Graphs representing the gene expression of A. MDR 1; **B.** ERK1 and **C.** ERK2 in TA and SRCC patients. Gene upregulation after NACT in **D.** MDR1; **E.** ERK1 and **F.** ERK2. **G.** The gel representing RT-PCR analysis of Sorcin, MDR1, and ERK1/2 in patients underwent upfront surgery and NACT. P1-2= Patient 1 and 2; CRs= complete responders and NRs= non-responders. Values significant at p<0.05, p<0.001, p<0.0001.

4.7.2. Protein expression analysis

The immunostaining of P-gP and ERK1/2 exhibited a similar expression pattern to Sorcin in both TA and SRCC cases. Among upfront surgery recipients, thirty-eight patients displayed strong P-gP staining (H-score = 3+ & 4+), while eighteen NACT-treated patients showed strong staining in more than 51% of cells (H-score = 4+).

Furthermore, the majority of patients found to have partial intermediate ERK1/2 expression (IHC-score = 2+). Notably, nine out of ten NACT-treated patients exhibited intermediate to strong ERK1/2 staining (>50 %; H-score = 3+). Among upfront surgery recipients, approximately 52% displayed intermediate P-gP staining (H-score = 2+). However, following NACT treatment, the intensity level significantly increased, with 90% of TA patients showing higher P-gP (H-score = 4+) and ERK1/2 (H-score = 3+) expression (p = 0.005; p = 0.001, respectively), similar to Sorcin (H-score = 3; p = 0.034) **(Figure 19)**.

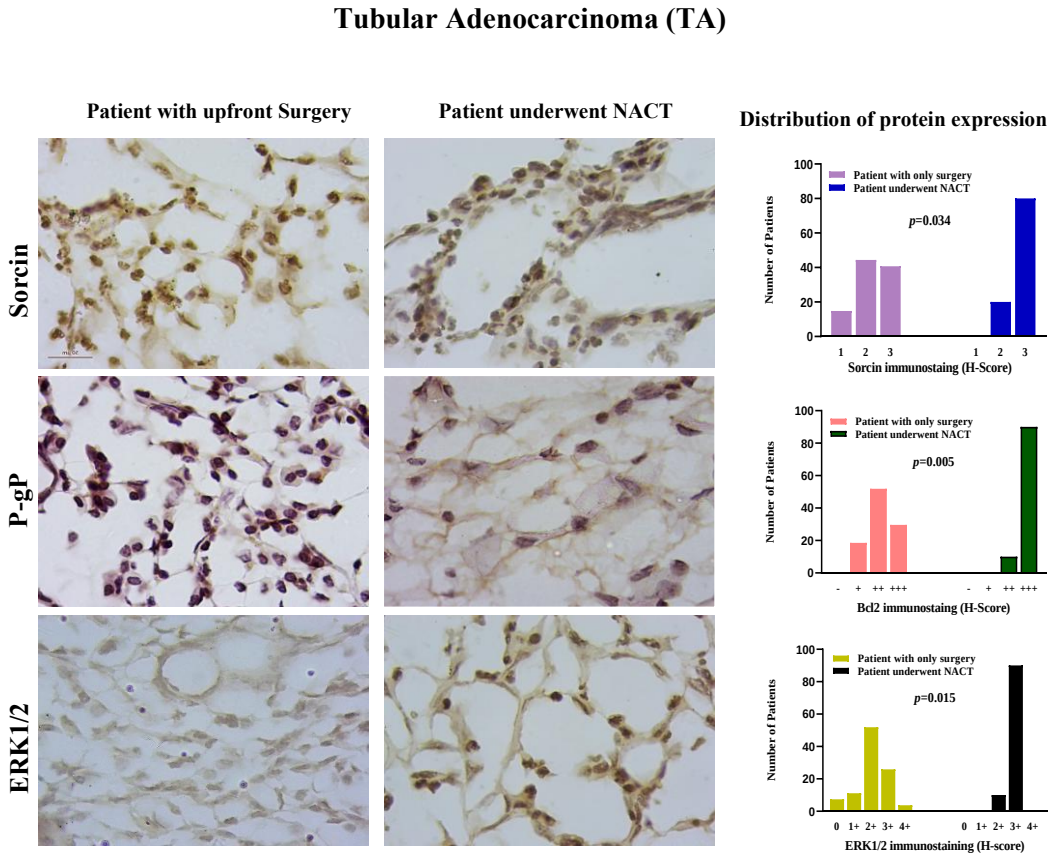


Figure 19: Immunohistochemistry staining of Sorcin, P-gP, and ERK1/2 of patients received upfront surgery and NACT in TA cases (left). The graphs

(right) represent the distribution of TA patients in two groups, upfront surgery and NACT.

In SRCC cases, the expression pattern of P-gP differed from that observed in TA cases. Notably, P-gP intensity was significantly downregulated in 44% of NACT-treated patients, while it was upregulated in approximately 40%. In contrast, P-gP expression was minimal among upfront surgery recipients.

Additionally, ERK1/2 expression (H-score = 3+ and 4+) was detected in more than 40% of upfront surgery patients, whereas 83% of NACT-treated patients exhibited elevated ERK1/2 expression (H-score = 4+).

On the other hand, the trend of Sorcin intensity (H-score = 3) in SRCC cases following NACT closely resembled that observed in TA cases under the same treatment conditions (**Figure 20**).

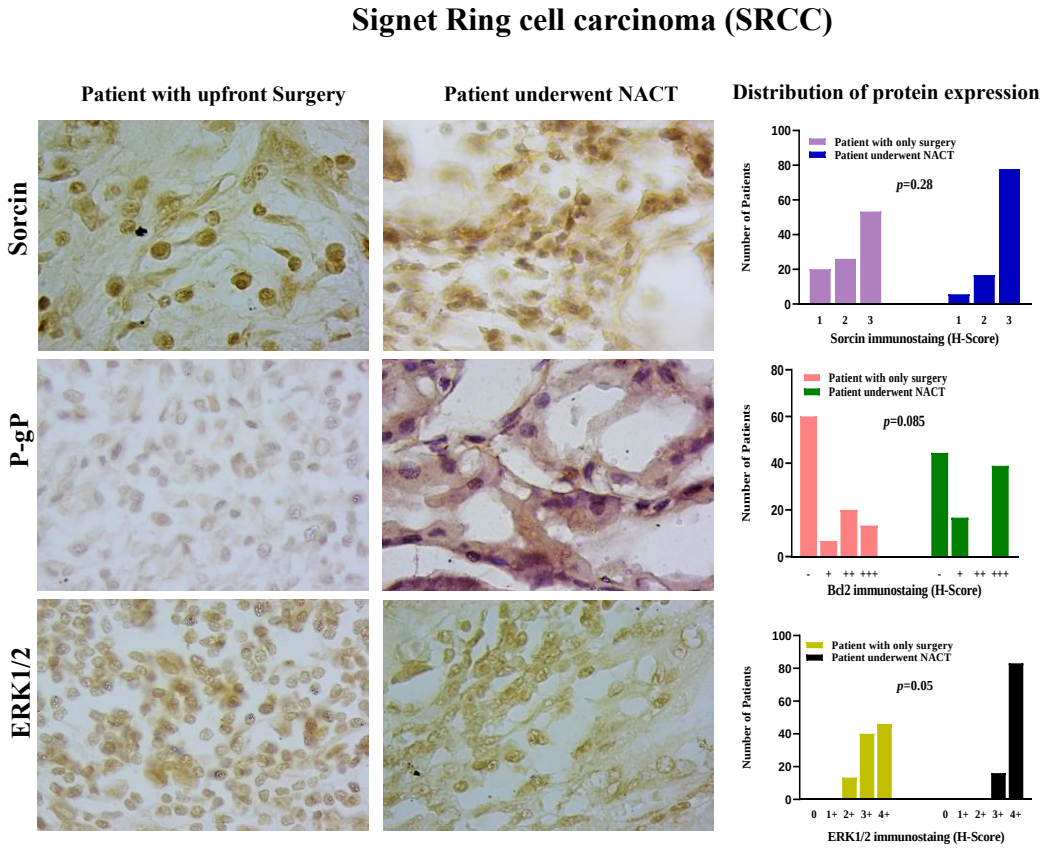


Figure 20: Immunohistochemistry staining of Sorcin, P-gP, and ERK1/2 of patients received upfront surgery and NACT in SRCC cases.

4.8. Assessment of MDR phenotype using rhodamine 123 accumulation assay among GC patients

The treatment response (CRs, PRs, and NRs) in patients, as determined by CECT reports, was further analyzed by assessing P-gP activity using the Rh123 accumulation assay. Normal adjacent tissues (control) exhibited the highest Rh123 accumulation (60.7%), indicating higher drug retention, whereas upfront surgery cases showed a reduced Rh123 accumulation (34.4%), suggesting increased drug efflux (**Figure 21 A**). Following NACT, CRs displayed higher Rh123 intensity (53.5%), while NRs exhibited the lowest accumulation (31.2%), indicating enhanced multidrug resistance (**Figure 21 B**).

Statistical analysis revealed significant differences among the groups ($p<0.005$), with NRs showing Rh123 intensity below 50%, significantly lower than in CRs, upfront surgery cases, and normal adjacent tissues (control) (**Figure 21 C**).

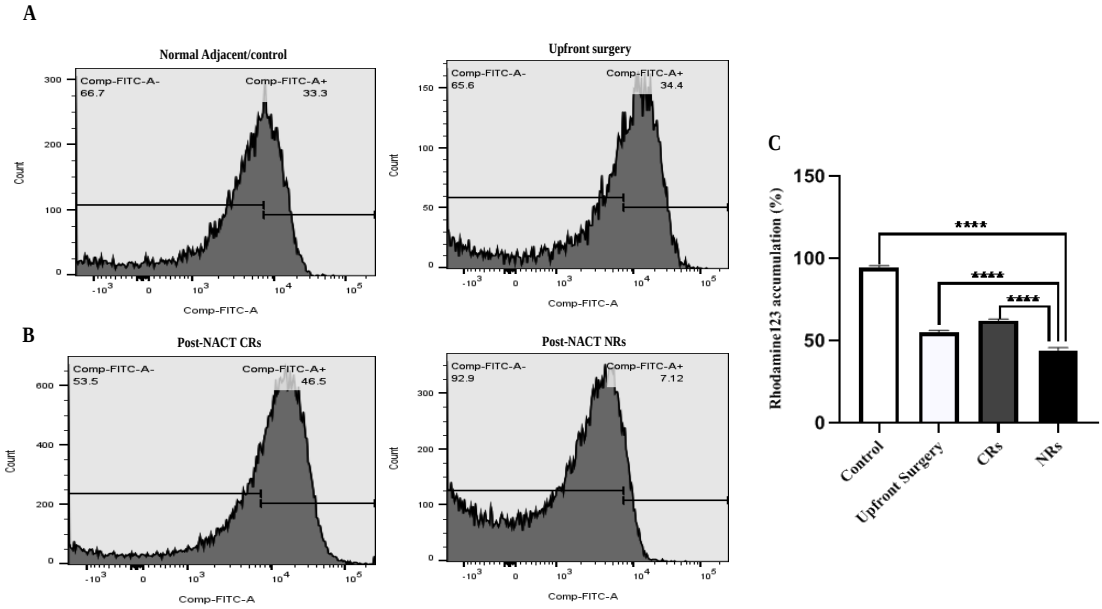


Figure 21: Rhodamine123 intensity in normal adjacent and GC patients A. upfront surgery, **B.** complete responders (CRs) and non-responders post-NACT. **C.** Graph representing the percentage of rhodamine retention in cells of GC patients. P-value is significant at *p<0.05, ***p<0.001, ****p<0.0001.

4.9. Sorcin expression in relation to treatment response along with P-gP and ERK1/2

The immunohistochemistry revealed distinct expression patterns of Sorcin, P-gP, and ERK1/2 among CRs and NRs in GC patients undergoing NACT. Both upfront surgery and NACT cases predominantly exhibited strong Sorcin expression (H-score = 3). P-gP expression varied, with upfront surgery patients exhibiting intermediate staining (H-score = 2+ & 3+) in 20–50% of cells, while NACT cases showed strong P-gP expression in more than 50% of cells (H-score = 4+). In upfront surgery cases, most patients displayed partial to intermediate ERK1/2 staining (H-score = 2+), whereas NACT recipients demonstrated diffuse strong staining (H-score = 4+) (**Figure 22 A**).

In the context of MDR phenotype, NACT patients classified as CRs showed a significant downregulation of Sorcin (p = 0.0001), P-gP (p = 0.0005), and ERK1/2 (p = 0.003), suggesting a reduced MDR in therapy-responsive cases. In contrast, NRs exhibited significantly higher expression levels of these markers, indicating a strong association between Sorcin, P-gP, and ERK1/2 with chemoresistance (**Figure 22 B**).

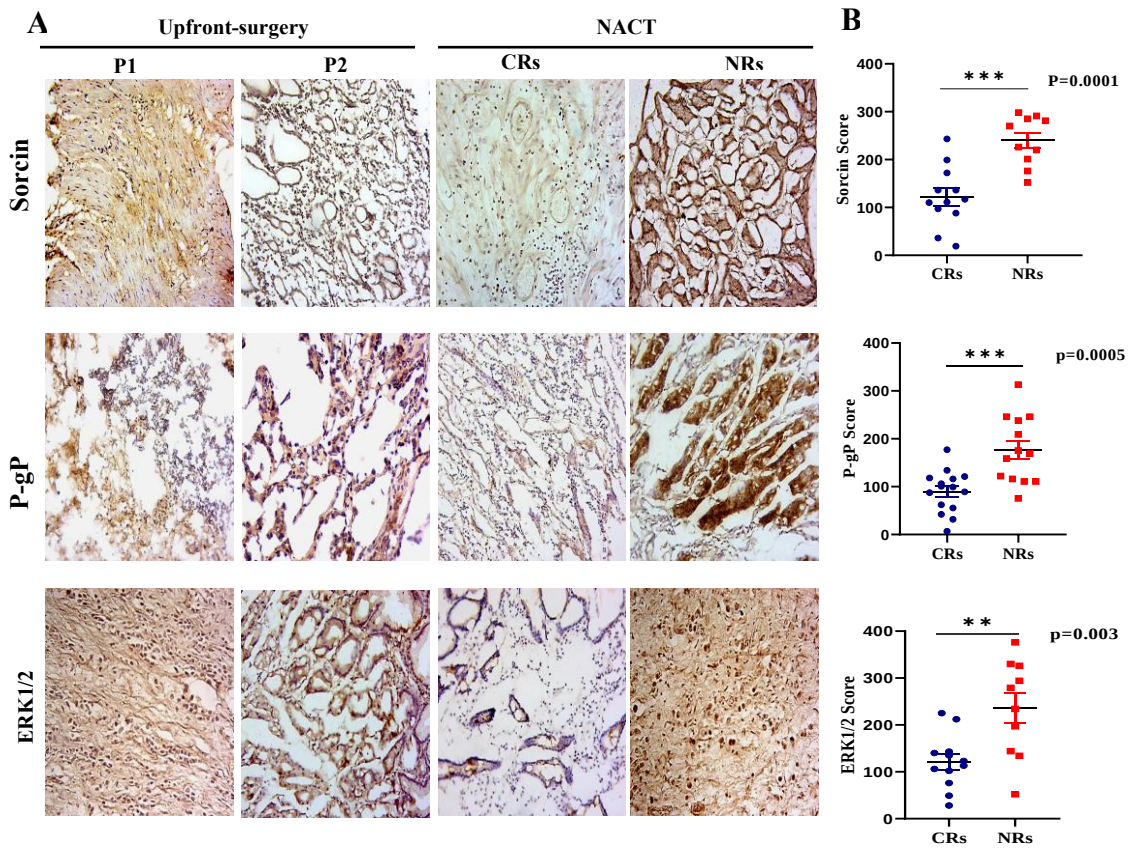
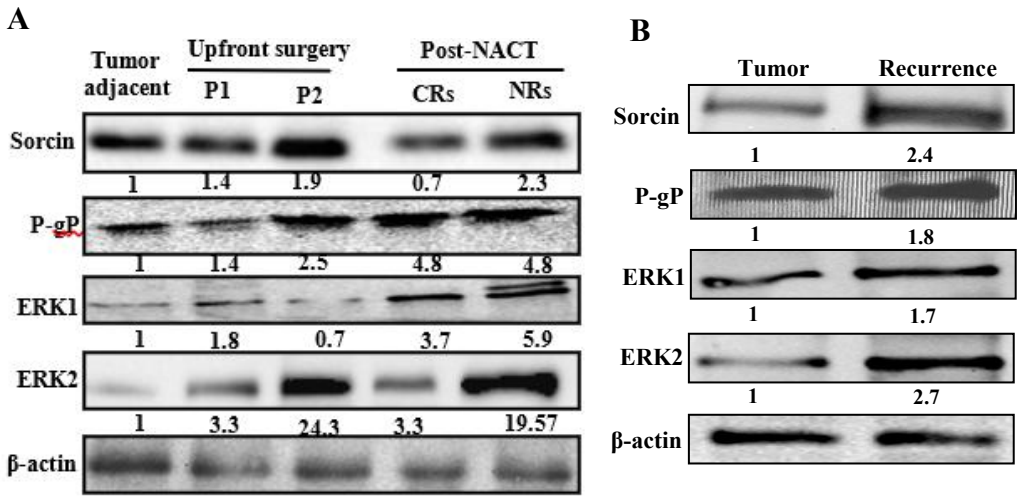


Figure 22: A. Immunostaining of Sorcin, P-gP and ERK1/2 in patients with upfront surgery and NACT (CRs and NRs). **B.** The representative graphs depicting significant difference between CRs and NRs for Sorcin, P-gP and ERK1/2 expression. *p<0.05, **p<0.01, ***p<0.001, P1&2= patients 1&2; CR=complete responders; NRs= non-responders.

Western blot analysis further validated these findings, demonstrating that NR patients exhibited significant upregulation of Sorcin (2.3-fold), along with increased expression of P-gP (4.8-fold) and ERK1/2 (5.9- and 19.5-fold, respectively). Interestingly, CRs shown to downregulate the Sorcin expression by 70% unlike P-gP and ERK1/2 (**Figure 23 A**). The results highlighted that Sorcin expression distinctly differentiated CRs from NRs, even more prominently than P-gP expression. Notably, Sorcin was found to be overexpressed (>2-fold) in NACT-treated recurrent cases identified as NRs. Additionally, both P-gP and ERK1/2 showed a >1-fold increase in expression compared to adjacent normal tissues (**Figure 23 B**).

Furthermore, Pearson correlation analysis revealed a moderate positive correlation between Sorcin and ERK1/2 (r = 0.50, p = 0.009) and Sorcin and P-gP (r = 0.43, p = 0.042) in NACT subsets, suggesting a probable functional regulatory interaction among these proteins (**Figure 23 C-D**).



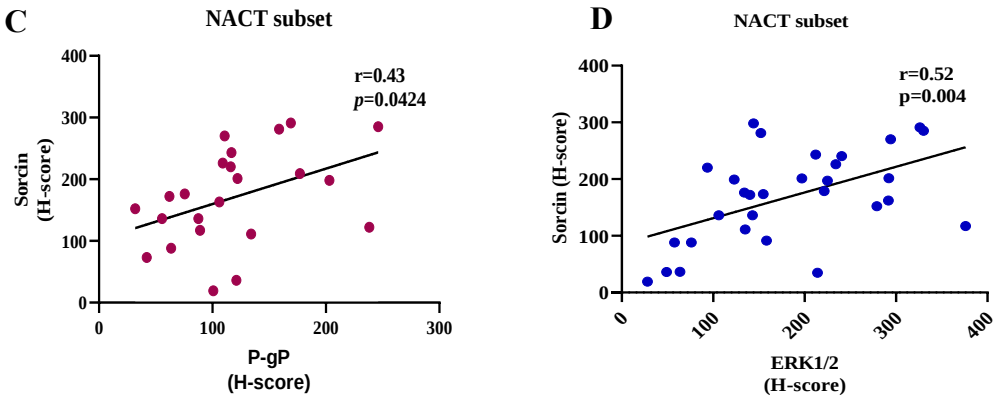


Figure 23: A. Western blot data representing Sorcin, Pg-P and ERk1/2 expression. **B.** Sorcin, P-gP and ERK1/2 expression in NACT recurrent cases**. C&D.** Pearson correlation analysis plotted for Sorcin with Pg-P and ERK1/2 in NACT subsets. *p<0.05, **P<0.01, P1 &2= patients 1&2; CRs=complete responders; NRs= non-responders.

4.10. Interaction of Sorcin with P-gP and ERK1/2 in GC Patients

Further, to examine the functional relationship between the proteins, protein-protein interaction analysis was performed between Sorcin and P-gP, as well as Sorcin and ERK1/2. Sorcin as a bait protein shown to give prominent band at the same molecular weight of ERK1/2 and P-gP. Remarkably, the result demonstrated that Sorcin-immunoprecipitated fraction showed high interaction against P-gP and ERK (**Figure 24).**

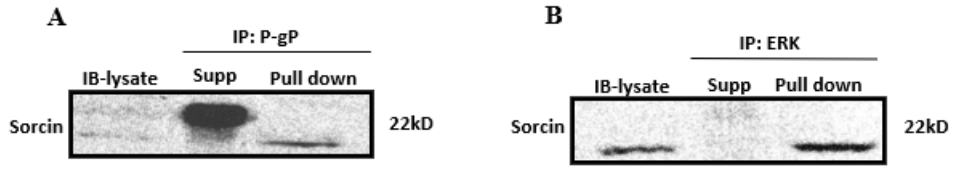


Figure 24: Co-immunoprecipitation showing interaction between A. Sorcin-P-gP and B. Sorcin-ERK in GC patients.

4.11. Impact of Sorcin on overall survival and hazard risk

The patients with high Sorcin level had OS of 13 months [95% CI: 2.38-14.36] with log-rank (Mantle-Cox) p-value of 0.394 than those with low Sorcin expression (**Figure 25 A**). Cox-proportional hazard analysis showed HR of 1.32 [95% CI: 0.681-2.57] for high Sorcin expressing individuals (p=0.408).

Furthermore, in TA cases, the mean OS of patients exhibiting higher Sorcin expression was 18 months [10.93-25.5; p = 0.220] and HR of 0.575 [0.58-2.88; p = 0.365] (**Figure 25 B**). The SRCC cases with higher Sorcin expression showed mean shorter OS of 10 months [6.62-15.01; p = 0.065]. The univariate cox-proportional hazard analysis anticipated non-significant HR of 2.055 [0.909-4.645; *p* = 0.084] in SRCC (**Figure 25 C**).

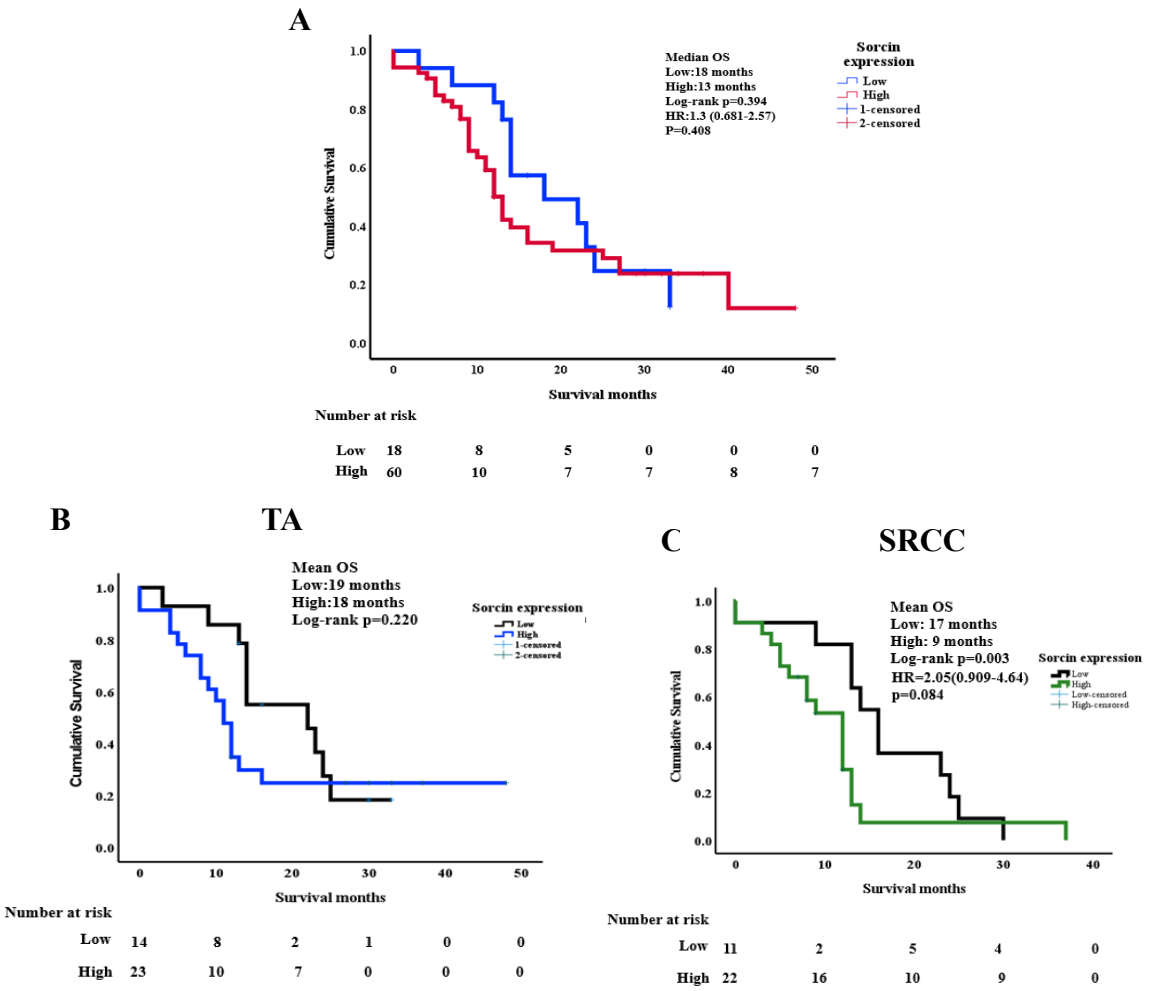


Figure 25: Overall survival with Sorcin. A. Kaplan meier analysis of patients with Sorcin expression in overall GC population **B&C.** patients with TA and SRCC.

Furthermore, it has been noticed that clinicopathological characteristics like epigastric lump, serosal invasion, tumor grade, and pTNM stage in SRCC significantly associate with Sorcin and indicating higher hazard risk (HR>1; p<0.05) as compared to TA cases **(Figure 26)**.

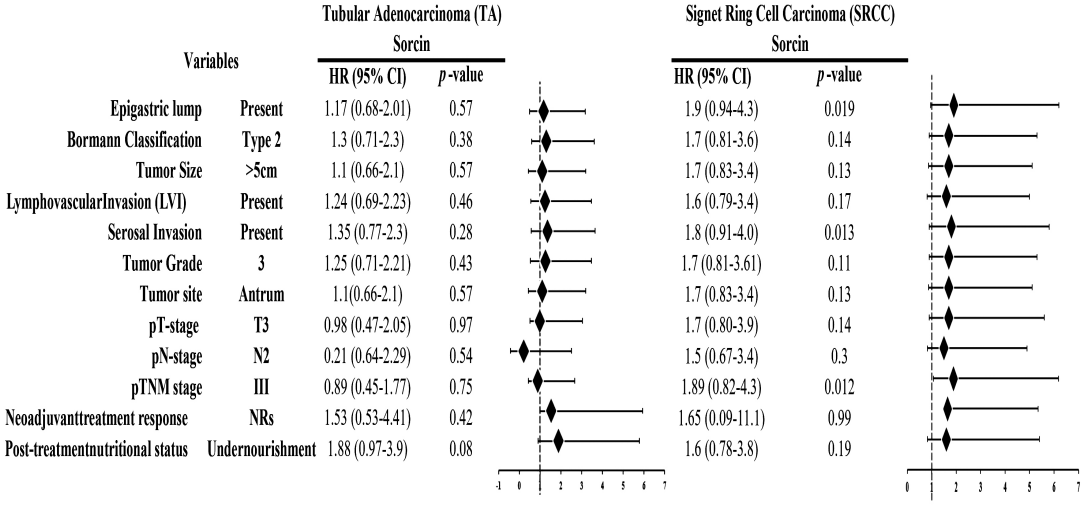


Figure 26: Clinicopathological parameters with Sorcin presented risk of hazard of the disease in TA and SRCC cases.

The clinical findings of this study were further validated *in vitro* to confirm the role of Sorcin in MDR and to delineate its interplay with P-gP and ERK1/2.

4.12. Development of drug resistant AGS cell lines against 5FU, oxaliplatin and docetaxel

To develop resistance, AGS cells were exposed to increasing concentrations (0, 6.25, 12.5, 25, 50, and 100 nM) of 5FU, oxaliplatin, and docetaxel. The acquired resistance in the resulting resistant cell lines—AGS/5FUR, AGS/OxaR, and AGS/DoceR—was assessed using MTT assay, Rh123 accumulation assay, and P-gP expression analysis.

The IC_{50} values for drug-sensitive AGS cells were determined to be 10.81 nM for 5FU, 15.94 nM for oxaliplatin, and 13.56 nM for docetaxel. Resistance was successfully induced at a concentration of 1 nM for each drug. The AGS/5FUR, AGS/OxaR, and AGS/DoceR cells exhibited a statistically significant increase in IC_{50} values compared to drug-sensitive AGS cells, with values of 19.52 ± 0.22 nM, 32.56 ± 0.018 nM, and 25.05 ± 0.011 nM, respectively. The resistance index (RI), calculated as the ratio of the IC_{50} of resistant cell lines to that of wild-type cell lines, was 1.8 for 5FU, 2.0 for oxaliplatin, and 1.8 for docetaxel, indicating an overall increase in drug resistance by more than 1.8-fold. Cell viability analysis further revealed that, upon treatment with their respective

drugs, resistant cells exhibited significantly higher viability compared to drug-sensitive AGS cells ($p < 0.05$) (**Figure 27**).

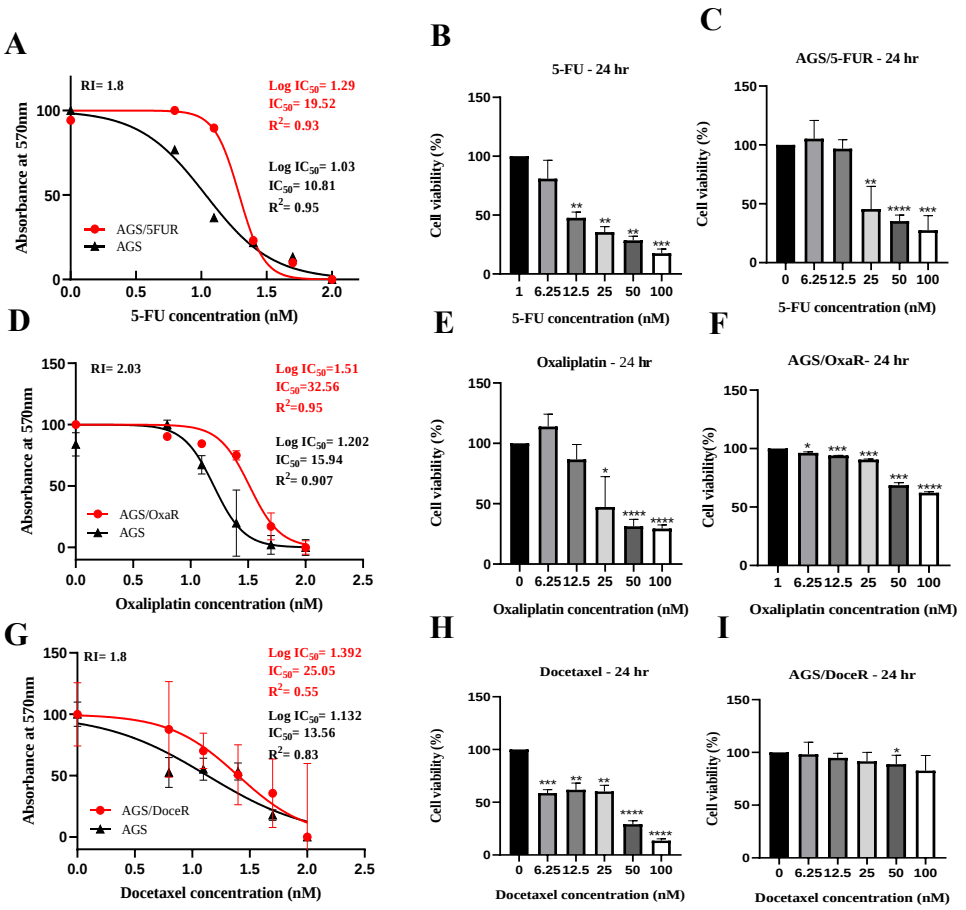


Figure 27: Response of drug-sensitive and drug-resistant cells to treatment with various concentrations of 5FU, oxaliplatin, and docetaxel. The MTT assay, cell viability values and cell morphology obtained from drug sensitive AGS and (**A-C**) 5FU-resistant (AGS/5FUR) (**D-F**), oxaliplatin-resistant (AGS/OxaR), and (**G-I**) docetaxel-resistant (AGS/DoceR) cells were normalized to the untreated sample. The data are presented as the mean ± SE; * $p < 0.05$; ** $p < 0.01$, ***$p<0.001$, ****$p<0.0001$.

Additionally, the cells adopted unique characteristic morphologies on resistance. AGS cells exhibit epithelial-like morphology characterized by tightly packed polygonal and giant adherent cells with distinct borders arranged in sheet like fashion. Whereas, resistant cells became elongated to elliptical in shape, notably with spiked ends suggesting invasive growth (**Figure 28**).

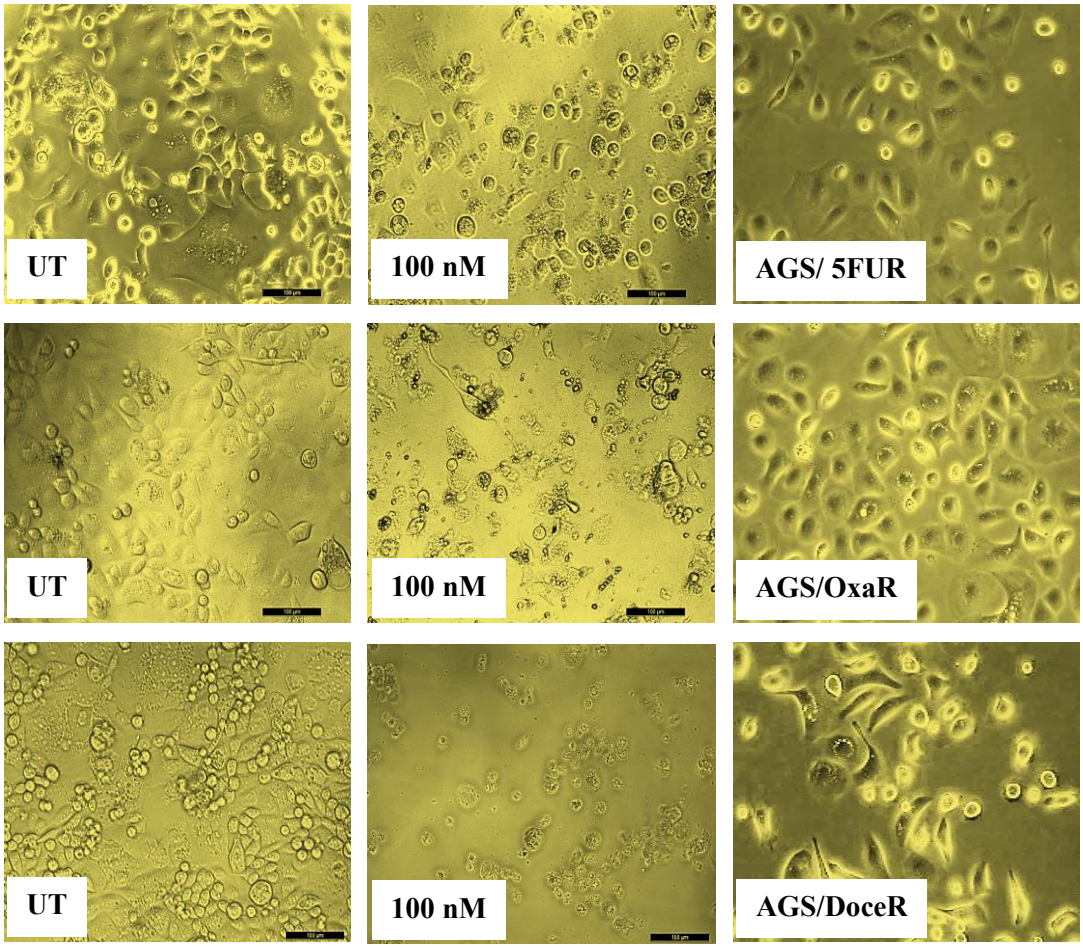


Figure 28: Phase-contrast microscopy images (40×) showing cell morphology in response to various concentrations of 5FU, oxaliplatin, and docetaxel.

4.13. Evaluation of acquired drug resistance phenotype in AGS/5FUR, AGS/OxaR, and AGS/DoceR Cells Using Rh123 Accumulation Assay

The Rhodamine 123 (Rh123) accumulation assay results confirm the presence of an MDR phenotype in AGS drug-resistant cell lines (AGS/5FUR, AGS/OxaR, and AGS/DoceR). Flow cytometry histograms show that control AGS cells exhibit high Rh123 fluorescence intensity (graph shifted to right), indicating low drug efflux activity. In contrast, drug-resistant cells display a significant reduction in Rh123 accumulation (graph shifted left side), suggesting enhanced drug efflux mediated by P-glycoprotein (P-gP). Treatment with Verapamil, a known P-gP inhibitor, restores Rh123 accumulation in resistant cells, shifting the fluorescence intensity closer to control levels, thereby confirming that the efflux activity is P-gP-dependent (**Figure A-J**). The bar graph quantitatively supports these findings, where resistant cell lines show

significantly reduced Rh123 accumulation compared to AGS-UT cells, with statistically significant differences ($p < 0.05$, $p < 0.01$, $p < 0.001$). However, upon Verapamil treatment, Rh123 retention increases, further validating that P-gP overexpression contributes to the MDR phenotype (**Figure 29 K**).

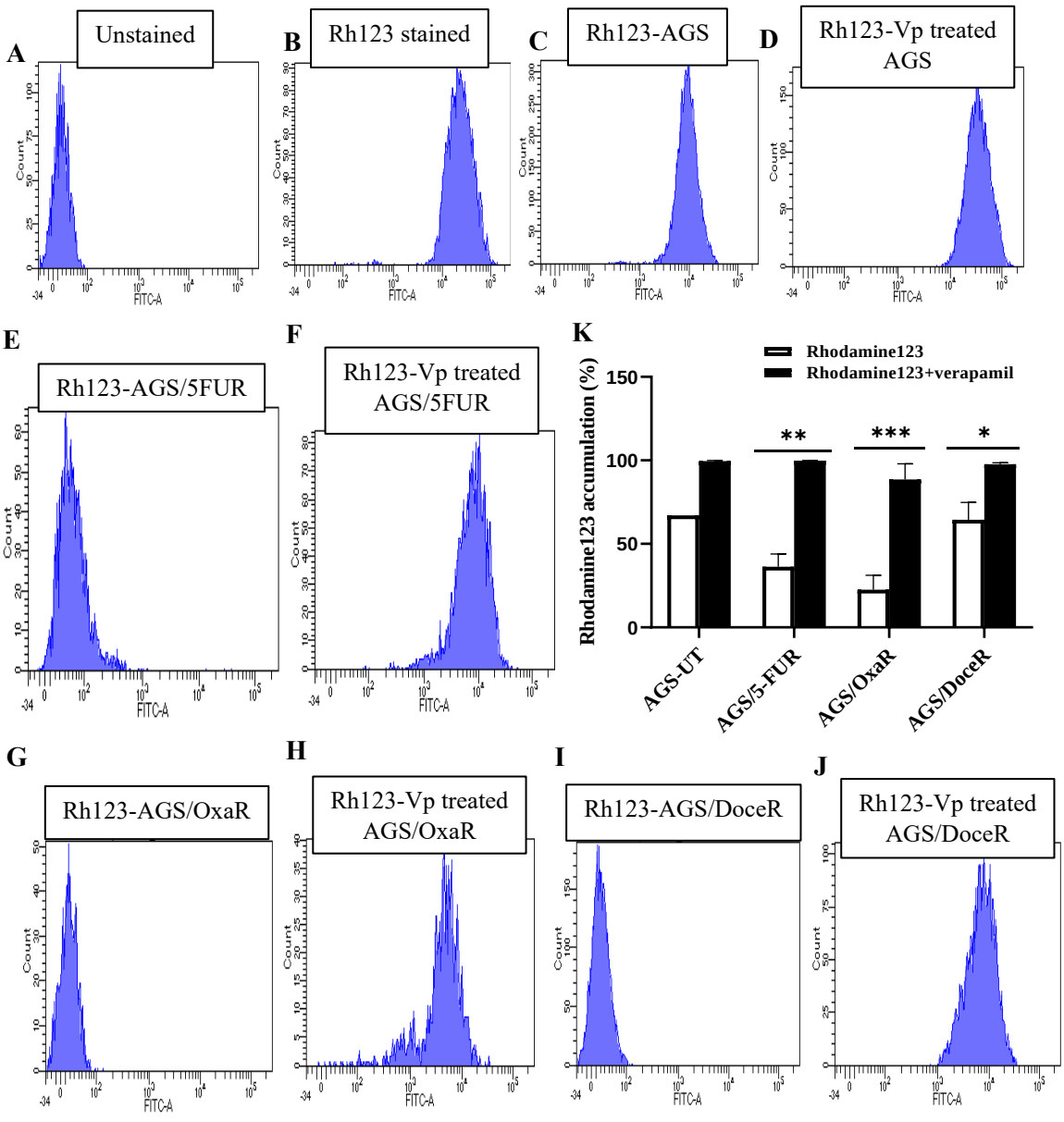


Figure 29: Flowcytometry depicted the intracellular rhodamine accumulation in drug resistant cells and compared to the verapamil treated cells. A. unstained, **B.** Rhodamine123 stained AGS cells, **C.** Rh123 and verapamil treated AGS cells, **D.** Rh123 and verapamil treated AGS/5FUR cells, **E.** Rh123 and verapamil treated AGS/OxaR cells, **F.** Rh123 and verapamil treated AGS/DoceR cells. The graph represents rate of Rh123 accumulation in cells; * $p < 0.05$, ** $p < 0.01$, ***$p<0.001$, ****$p<0.0001$.

4.14. Sorcin expression in HEK293, AGS and AGS/5FUR, AGS/OxaR and AGS/DoceR cells

Differential expression of Sorcin in normal and cancerous in response to chemotherapeutic drugs has been demonstrated *in vitro*. Sorcin expression is notably adequate in HEK293 normal cells, whereas AGS gastric cancer cells exhibit significantly lower expression—approximately 70% less. To understand the impact of chemotherapy on Sorcin expression, AGS cells were exposed to increasing concentrations of 5FU, oxaliplatin, and docetaxel, revealing distinct response patterns. 5FU treatment beyond 12.5 nM significantly induced Sorcin expression, with the highest upregulation observed at 25 nM (around 2-fold), after which expression plateaued at 50 and 100 nM. Oxaliplatin exhibited a biphasic effect, initially enhancing Sorcin expression from 6.25 nM to 25 nM (~3-fold), followed by a marked decline at 100 nM. In contrast, docetaxel treatment led to a differential upregulation of Sorcin across all tested concentrations, indicating a dose-independent increase, it upregulated at 6.25 nM and 12.5 nM while became constant through the remaining concentrations.

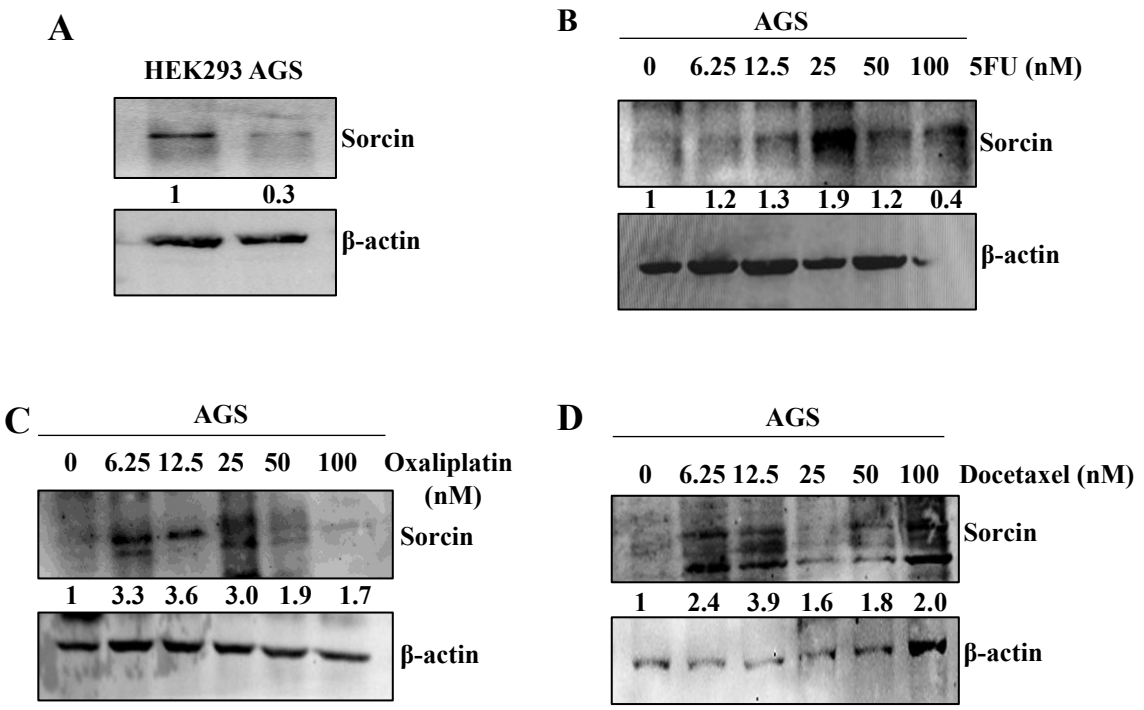


Figure 30: Protein expression of Sorcin in normal, AGS and drug-resistant cells. The data are presented as the mean ± SE showing the relative expression of Sorcin.

4.15. Correlation of Sorcin with Pg-P and ERK1/2 in drug sensitive and resistant AGS cells

The drug-resistant cell lines—AGS/5FUR, AGS/OxaR, and AGS/DoceR—exhibited a significant overexpression of Sorcin, with respective increases of 3.6-, 3.4-, and 6.5-fold at the mRNA level and 2.8-, 5.3-, and 5.7-fold at the protein level. Notably, AGS/DoceR cells demonstrated the highest Sorcin expression among the three resistant variants. Additionally, P-gP expression was elevated at both the mRNA and protein levels in resistant cells, confirming the acquisition of drug resistance. Interestingly, AGS cells treated with a combination of 5FU, oxaliplatin, and docetaxel exhibited a remarkable 17.6-fold increase in Sorcin expression, surpassing that of P-gP. Furthermore, ERK1/2 expression mirrored the trend of Sorcin upregulation, suggesting a possible functional correlation between these proteins in mediating drug resistance **(Figure 31 A-C).**

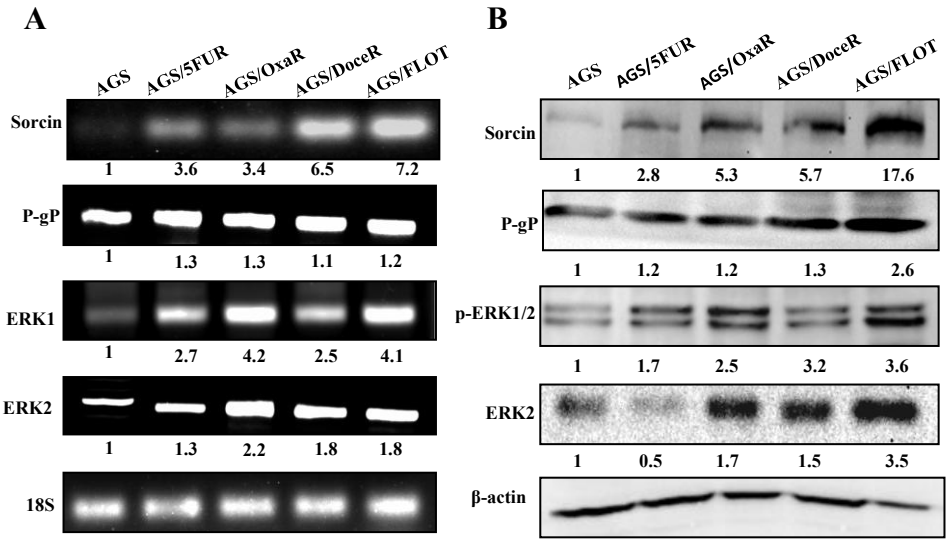


Figure 31: A. RT-PCR and **B.** Western blot analyses depict the expression of RAS/RAF/ERK pathway proteins at various concentrations following treatment with 5FU, oxaliplatin, and docetaxel in drug sensitive and resistant AGS cells. The data are presented as the mean ± SE showing the relative expression of Sorcin.

4.16. Sorcin suppression attenuates ERK1/2 expression in drug sensitive and resistant gastric cancer cells

Sorcin-targeted siRNA was successfully transfected into both drug-sensitive AGS cells and resistant AGS/5FUR, AGS/OxaR, and AGS/DoceR cells. The silencing of Sorcin led to a significant downregulation of P-gP and ERK1/2 expression ($p<0.05$), highlighting its role in MDR. Compared to drug-sensitive AGS cells, siRNA administration in resistant cells completely inhibited Sorcin expression at varying concentrations (30 nM and 40 nM). Among the resistant variants, 90% depletion of Sorcin resulted in a 90% reduction of ERK1/2 expression in AGS/5FUR cells and 70% in AGS/DoceR cells, whereas AGS/OxaR cells showed comparatively less attenuation. This suggests an intricate interplay between Sorcin and ERK1/2, influencing MDR. Additionally, the downregulation of P-gP upon Sorcin silencing in both drug-sensitive and resistant cells further support its regulatory association with MDR mechanisms **(Figure 32)**.

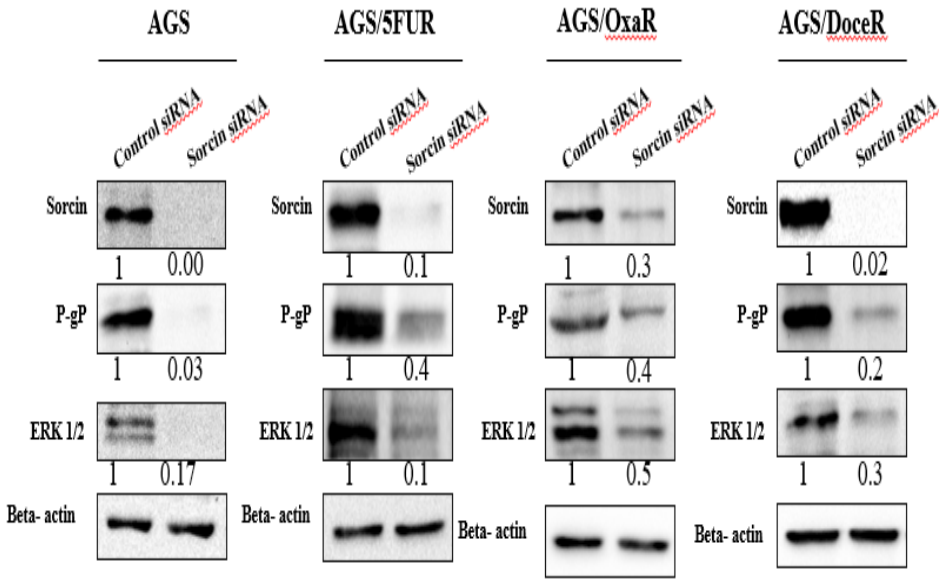


Figure 32: Western blot analysis showing the expression of P-gp and ERK pathway proteins following treatment with 5FU, oxaliplatin, and docetaxel in drug-resistant AGS cells. The data are presented as the mean ± SE.

CHAPTER 5
DISCUSSION

The present study observed that gastric cancer is associated with dominant grade III SRCC tumors which are often difficult to manage due to the aggressive features like large tumor size, high lymph node invasions, and lymphovascular serosal invasion. Neoadjuvant FLOT chemotherapy and malnutrition were significant factors contributing to poor overall survival, majorly within middle aged patients. Sorcin was found to be elevated at transcriptional and translational levels in GC, particularly in SRCC, where it was overexpressed with P-gP and ERK1/2. In post-NACT patients, Sorcin levels were increased in NRs to FLOT while decreased in CRs. Clinical validation revealed its association with the MDR phenotype and its interaction with ERK1/2 and P-gP. There are notable differences where all cancer patients in the clinical setting had Sorcin expression, while AGS cells demonstrated very low which was increased after treatment with 5FU, oxaliplatin, and docetaxel, however, at different concentrations. These observations suggest the intricate involvement of Sorcin in MDR as well as its association with ERK1/2 and P-gP.

Majority of GC cases in the cohort were adenocarcinomas, predominantly of the TA and SRCC subtypes. The demography has shown male dominance with median age of 52±11.35 years. Most individuals were large consumers of mix diet containing high salt, smoked meat, or raw fish, and preserved food. Lifestyle factors, including smoking, tobacco use, and alcohol consumption, were frequent, along with a personal history of peptic disorders. Furthermore, consuming preserved foods, processed grilled or barbecued meat or fish, drinking alcoholic s per day (> 45 g ethanol/day), high salt and obesity increase the risk of GC in the North-East part of India, parts of eastern Asia, Europe and the West (WCRF/AICR 2018**;** Behera et al., 2023; Chakraborty et al., 2023; Morgan et al., 2023; Scherubl et al., 2021; Song et al., 2018; Bhat et al., 2015). Several epidemiological studies have reported decreasing incidence of GC and increasing number of SRCC cases in older age group, males residing in urban setup (Altay et al., 2020; Grantham et al., 2023).

Herein, the patients had a past history of peptic disorder, gastric ulcer, chronic gastritis, and gastric lump. Initially majority of the patients experienced severe abdominal pain, and progressive weight loss at the time of diagnosis. Similar findings were also identified these parameters as the most common symptoms at initial diagnosis (Ramachandran et al., 2024). Thus, presence of these symptoms highlights the impact of advanced GC on quality of life of patients.

Furthermore, the clinicopathological features of GC revealed that the patients had an ECGO performance score 2 or 3, tumor growth at the “antrum”, with tumor sizes >5 cm. The radiological findings have shown substantial stomach wall thickening, in line with other studies (Bhattarai et al., 2021; Kim et al., 2019). The histopathological analysis exhibited distinct cellular characteristics, with most patients exhibiting “fungating carcinoma” (Type II) features as per Bormann's classification and nearly equal intestinal and diffuse type features as compared to diffuse type according to Lauren's classification. Supporting studies reported a 45.2% intestinal type, 43.2% diffuse type, and 7.3% mixed GC subtypes types among the patients (Dominguez et al., 2024). Additionally, WHO classification, in our study characterized poorly differentiated carcinoma associated with SRC cells. This finding aligns with other data, showing increased trend of SRCC, particularly among middle-aged males (Ghosh et al., 2021; Wang et al., 2023). Moreover, poorly differentiated cases (73%) showed the presence of LVI, pT3 tumor extension, pN2/3ab lymph node involvement, and pTNM stage IIIA/B were prominently observed in the biopsy samples. A subset of patients also exhibited serosal and perineural invasions. Distinct characteristic differences were observed between TA and SRCC cases. SRCC was more frequently diagnosed among younger females, while TA was more prevalent in middle-aged males. Despite shared traits, including a familial history of gastric ulcer, pT3 tumor extent, pN2-3 lymph node invasion, and the presence of LVI, the subtypes showed distinguished differences. TA was significantly associated with smoking, a personal history of peptic disorder, and smaller tumor sizes unlike SRCC. Differences in clinicopathological features between these subtypes have also been reported, highlighting their distinct biological and clinical behaviour (Efared et al., 2020; Arai et al., 2019).

This study describes the treatment protocol of the cohort including D2-lymphadenectomy with distal gastrectomy, followed by adjuvant CAPOX chemotherapy administered across all age groups. For patients exceeding 5 cm tumor sizes, a regimen of four cycles of neoadjuvant FLOT chemotherapy was recommended. The treatment response of the cohort, observed the overall response rate (ORR) comprising complete responses (CRs) and partial responses (PRs) in 43.6% patients, while the remaining patients were non-responders (NRs), indicating the limited efficacy of the current therapeutic regimens in a subset of patients. Remarkably, the majority of patients in the overall cohort were NRs, both after ACT (55.1%) and NACT (58.6%), failing to show significant tumor regression presenting limitations in RECIST criteria. Consistent with previous studies, where RECIST criteria failed to effectively identify patients progressing to metastatic disease or stratify them into subsets with distinct survival outcomes, highlighted that TNM re-staging demonstrated a strong prognostic value (Sando et al., 2023; Yang et al., 2023).

The study also highlighted the significant burden of post-treatment complications, affecting 66.7% of the patients, mostly due to malnutrition (61.5%), followed by nausea and vomiting. This high incidence highlights the need for comprehensive supportive care strategies to mitigate treatment-associated morbidity and improve patient outcomes. The study by Zheng et al. reported comparable evidence associated with severe postoperative complications, recurrence, and mortality in patients with GC (Zheng et al., 2024). In contrast, Park et al observed that totally laparoscopic total gastrectomy followed by NACT significantly reduced the incidence of grade I pulmonary complications and provided improved quality of life in terms of dysphagia, pain, eating, and odynophagia compared to totally laparoscopic total gastrectomy for patients with clinical stage I gastric cancer (Park et al., 2021). Furthermore, a recent randomized study further emphasizes the benefits of prehabilitation including improved physical capacity before surgery, reduced non-compliance with neoadjuvant treatment and decreased incidence of postoperative complications by 60% with enhanced quality of life compared to standard care (Bausys et al., 2023).

Furthermore, the 5-year OS of the cohort was 66.7%, with 33.3% of patients censored. The mean OS for the population was 19.7 months, and the recurrence rate was 14%. Similarly, a study from North India reported a comparable mean survival duration of 20.01 months, aligning closely with the findings in this cohort (B AV et al., 2023; Chakraborty et al., 2023; Morgan et al., 2023).

Patients who experienced significant weight loss had a significantly lower OS of (18.3 months), along with a significantly higher hazard ratio, indicating a higher risk of poor outcomes. Furthermore, age-stratified survival analysis revealed significant differences among younger, middle-aged, and elderly patients, with older patients demonstrating a two-fold higher hazard risk and shorter OS, suggesting that older age is a significant factor contributing to poorer survival outcomes. According to a recent report, patients with non-metastatic gastric cancer (GC) showed an improved 5-year overall survival rate (Pavliou et al., 2023; Bouras et al., 2022).

In contrast to several other reports (Ji et al., 2024; Pradhan et al., 2023; Dhillon et al., 2018), our study identified lower survival months with no significant hazard risk associated with gender, dietary habits (mixed diet), or addiction habits. Interestingly, tumor growth in the antrum and larger tumor sizes—typically associated with poorer outcomes—were observed to correlate with poor survival in our cohort. This finding contradicts the established associations between these factors and survival reported in other studies (Bouras et al., 2022). Besides, there was no significant survival difference between intestinal and diffuse type tumor classification, whereas, poorly differentiated tumor with SRC cells associated with worse prognosis but didn't show impact on survival (Wong et al., 2021). Patients with pTNM stage III had an OS (16.5 months), with an HR of 1.24, indicating a marginally higher risk, though not statistically significant. In terms of other factors, overall tumor size, grade, PNI, and SI, all indicated HRs>1, suggesting a potential risk factor for worse survival outcomes. However, the lack of statistical significance indicates that these factors do not independently predict OS in this cohort. A retrospective study by Zhong et al. (2023) analysed a cohort where 81% of patients had diffuse-type tumors and 91% had poorly differentiated tumors, with 82% presenting at stage III–IV. The

median OS for the entire cohort was 23.3 months, while patients at stage III had a median OS of 40.6 months (Zhong et al., 2023). However, the correlation found no relationship between grade with worse survival on univariate or multivariate analysis, whereas other studies showed grade as an important factor that affects patient's overall survival (Paredes et al., 2021). High grade tumors are more aggressive and have higher risks of metastasis and mortality (Van et al., 2023).

Regarding treatment regimens, patients receiving NACT FLOT chemotherapy had a significantly lower mean OS (17.7 months) and a notably high HR of 23.5, indicating a significantly higher risk of poor survival outcomes. In contrast, patients undergoing 6 cycles of ACT chemotherapy had a better mean OS (22.5 months) and a significantly low HR of 1.26, suggesting that ACT may be more effective for improving survival in this cohort. Furthermore, undernourished patients, with OS of 16.49 months, and HR of 1.32, suggests a potential increased risk for poorer outcomes. These observations are similar to a study by a Indian researcher (Krishnamoorthi et al., 2023).

The current study observed that the choice of chemotherapy regimen emerged as a significant factor impacting the overall survival of GC patients. Preoperative FLOT receivers showed compromised survival months compared to postoperative CAPOX receivers. The chemotherapy response of the patients were heterogenous irrespective of the use of different chemotherapy regimen where OS of the patients decreases with increasing chemotherapy cycles. Most of them were non-responders with lower overall survival month in either chemotherapy group. Interestingly, the risk of death indicated by different response categories (CRs, PRs and NRs) has no significant distinction among them. Research conducted in Eastern India also reported improved survival in patients undergoing surgery compared to other modes (Krishnamoorthi et al., 2023). On the contrary, perioperative FLOT and modified version of FLOT shown improved response and survival than other chemotherapeutic regimens in patients from different regions of India and other parts of the world (Ramaswamy et al., 2023; Tokunaga et al., 2023; Bhargava et al., 2022; Wang et al., 2019). The disparity in the observation in our cohort might be due to older

age, advanced tumor stage, epigastric lump (55.1%), anaemia (67.9%), weight loss (89.7%), type of gastrectomy (mainly total, proximal and subtotal; 31%) and post-operative complications (66.6%) like malnutrition. However, the OS of patients having SRCC appeared non-beneficial from FLOT treatment compared to surgery.

In our country, particularly in the state of West Bengal, where there are significant resource constraints in terms of access to tertiary healthcare for early diagnosis, adequate surgery, and chemotherapy, along with a shortage of qualified medical oncologists to manage chemotherapy-related toxicities (such as the highly toxic FLOT regimen), these treatments may not be as suitable as in other parts of the world. Studies highlighted the disparities in access to advanced cancer therapies in developing regions, emphasizing the need for improved healthcare infrastructure, socioeconomic factors which impact the accessibility and outcomes of cancer treatment in lower-income countries (Ringborg et al., 2024; Nicole Rose et al., 2023).

The pathological grading demonstrated significant correlation between tumor grade or age and gender highlighting the underpinning impact on GC progression. Notably, the association with epigastric lump and larger tumor size indicated that more aggressive tumors are likely to associate with the higher grades. Furthermore, its correlation with wall thickening suggests that advanced-grade tumors may exhibit deeper infiltration into the gastric wall. Pathological features, including Borrmann classification (Type-III), diffuse histological subtype, and SRCC, were strongly associated with higher grades, indicating a more aggressive phenotype of poorly differentiated tumors. Additionally, LVI, higher tumor extent, lymph node involvement, and post-operative undernourishment further contribute to worse clinical outcomes. Studies have demonstrated an implications and correlation between advanced tumour grades and signet-ring histology, neoadjuvant chemotherapy, sarcopenia, and other short-term and long-term outcomes in older patients (Zheng et al., 2024; Bhandare et al., 2024). Patients with high-grade tumors, particularly those exhibiting features such as SRCC, advanced TNM staging, and LVI, were further assessed with respect to Sorcin expression.

Expression of Sorcin was assessed especially in patients likely to exhibit drug resistance. The gene expression of Sorcin (SRI) significantly upregulated in GC (3.17-fold) as compared to adjacent normal tissues (1.76-fold) with a difference of 1.41 ± 0.505 (p=0.0077), indicating the pathological significance of Sorcin in tumorigenesis. Huan Tuo and colleagues also reported higher levels in GC tissues as compared to adjacent normal tissue (Tuo et al., 2017).

For the first time, expression of Sorcin was examined across the GC subtypes, revealing significantly higher gene expression in SRCC as compared to TA, further emphasizing its potential as a biomarker. Sorcin expression was also high across all grades of GC, most notable in poorly differentiated adenocarcinomas (p=0.0001), followed by well- and moderately-differentiated tumors (p=0.0007 and p=0.0005, respectively), associating it with tumor progression. The statistical correlation revealed that Sorcin share positive association with epigastric lump, Borrmann classification, tumor size, LVI, serosal invasion, pTNM stage III, pT3 tumor extent, pN2-3 lymph node status, and post-treatment undernourishment in both TA and SRCC cases.In line with these findings, Deng et al. (2010) demonstrated that Sorcin was overexpressed in 64.71% of GC patients and was significantly corelated with the depth of invasion, lymph node metastasis and TNM stage parameters (Tuo et al., 2017; Deng et al., 2010).

Further Sorcin expression was assessed in response to neoadjuvant FLOT chemotherapy, and observed significant upregulation both at mRNA and protein levels in NACT recipients compared to those who underwent upfront surgery. This notion suggests the chemotherapy induced Sorcin upregulation, potentially as part of a stress-adaptive response in tumor cells. Additionally, the upregulation is more evident in SRCC than TA cases. In support, other studies have also shown overexpression of Sorcin leads to MDR in cancers, further emphasizing its involvement with treatment response (Gong et al., 2014; He et al., 2011).

The study underlines dual role of Sorcin, first, as a prognostic marker linked to tumor aggressiveness, particularly in poorly differentiated and SRCC cases;

second, as a potential predictor of chemotherapy response, given its upregulation following NACT.

Furthermore, the prognostic value of Sorcin expression was evaluated in terms of overall survival in GC patients. The patients with high Sorcin expression had an OS of 13 months with no statistically significant difference in OS between those with high and low Sorcin expression, suggesting, in the general GC cohort, Sorcin expression alone may not be a robust predictor of overall survival. When analysed based on histological subtype, patients with high Sorcin expression in TA cases showed a mean OS of 18 months, and in SRCC patients, the mean OS was significantly shorter at 10 months, indicating a potential factor toward worse survival in SRCC patients.

Further analysis revealed that certain clinicopathological characteristics, including epigastric lump, serosal invasion, tumor grade, and pTNM stage in SRCC, were significantly associated with Sorcin expression ($p<0.05$), indicating a higher hazard risk (HR>1) in SRCC compared to TA cases. This suggests that Sorcin might serve as a potential prognostic indicator in SRCC, where its expression could correlate with more aggressive disease characteristics and a poorer prognosis. Few studies have shown that SRCC was associated with better prognosis (Tian et al., 2022; Imamura et al., 2016) while others have shown its association with worse prognosis (Voron et al., 2016). Moreover, an association of Sorcin with survival and prognosis in gastric adenocarcinoma patients has been reported with overall survival and progression free survival to be 35.9 months and 55 months, respectively (Shabnam et al., 2018).

Additionally, clinicopathological characteristics such as epigastric lump, serosal invasion, tumor grade, and pTNM stage in SRCC has shown significant relation with Sorcin expression, indicating a higher hazard risk ($HR > 1$; $p < 0.05$) compared to TA cases.

Subsequently, expression of P-gP and ERK1/2, related to drug resistance and cancer progression, has been analysed. The mRNA levels revealed significantly higher expression levels of MDR1 ($p=0.009$), ERK1 ($p=0.0003$), and ERK2

(p=0.079) in SRCC tissues compared to TA, supporting the trend of Sorcin expression. Fujimori et al., 2012 reported that p-ERK expression was evident in 61% of GC patients predominantly in SRCC cases, and served as an independent predictor of poor outcomes (Fujimori et al., 2012). P-gP expression in SRCC, has been reported in 41.7% of gastric cancer tissues, further supporting its role in drug resistance in SRCC. Additionally, NACT patients were analyzed for treatment response (CRs, PRs, and NRs) using the rhodamine accumulation assay. The analysis revealed that non-responders to NACT exhibited reduced intracellular rhodamine accumulation, due to P-gP-mediated efflux, confirming the status of the patient as NRs. However, despite this upregulation, MDR1 and ERK1/2 expression did not correlate significantly with treatment response, reflecting a lack of reliable association with chemotherapy efficacy. In contrast, Sorcin expression was significantly elevated in both SRCC tissues and NACT patients ($p < 0.05$), highlighting its potential role in chemotherapy response. When considering both upfront surgery and NACT, the overall objective response rate of the patients suggested that patients responded well to the treatment, consistent with findings from other studies (Tastekin et al., 2023; Sun J et al., 2021; Li Y et al., 2020).

A Pan-cancer analysis discussed the clinical significance and prognostic value of Sorcin in various cancers, however, precise distribution of Sorcin in the heterogenous GC population has not been highlighted (Zhang et al., 2021). Here, protein expression analysis has been assessed the treatment response in overall GC patients, which revealed a 2.3-fold increase in Sorcin expression and upregulation of P-gP (4.8-fold) and ERK1/2 (5.9- and 19.5-fold) in non-responders underwent neoadjuvant FLOT chemotherapy. While, significant downregulation of these markers observed in CRs (p=0.0001 for Sorcin, p=0.0005 for P-gP, and p=0.003 for ERK1/2). These findings suggest that elevated Sorcin, P-gP, and ERK1/2 expression in non-responders may be indicative of their contribution to multidrug resistance and poor chemotherapy response. Furthermore, in recurrence cases, elevated expression of Sorcin, P-gP, and ERK1/2 was observed. NACT recipients with recurrence exhibited a 2.4-fold increase in Sorcin expression at the protein level compared to P-gP (1.8-

fold) and ERK1 (1.7-fold), suggesting that the upregulation of these proteins may be associated with cancer recurrence and resistance to treatment.

Sorcin regulates ERK1/2 in EGFR signalling pathway contributing to cancer cell survival and drug resistance (Tito et al., 2023). ERK1/2, reportedly activate P-gP in the resistant cancer cells (Mun et al., 2020). Herein, Sorcin and ERK1/2 expression levels were correlated with P-gP. Pearson's correlation coefficient analysis demonstrated significant correlations between Sorcin, P-gP, and ERK1/2 in patients who underwent NACT, further supporting the hypothesis that these proteins are interconnected in mediating chemotherapy resistance. To further explore the molecular mechanisms underpinning role of Sorcin in GC progression and drug resistance, we investigated the protein-protein interactions between Sorcin and two key molecules, P-gP and ERK using co-immunoprecipitation, which demonstrated that Sorcin may physically interact with these molecules in GC tissues.

Next, *in vitro,* successful resistant AGS cell lines were established—AGS/5FUR, AGS/OxaR, and AGS/DoceR—following exposure to increasing exposure of 5FU, oxaliplatin, and docetaxel, respectively. Degree of resistance was evaluated in the resistance cells. The drug-sensitive AGS cells exhibited lower IC_{50} values for 5FU (10.81 nM), oxaliplatin (15.94 nM), and docetaxel (13.56). In contrast, the resistant cell lines demonstrated significantly elevated IC_{50} values: AGS/5FUR (19.52 ± 0.22), AGS/OxaR (32.56 ± 0.018), and AGS/DoceR (25.05 ± 0.011), indicating a marked reduction in drug sensitivity. The resistance index for 5FU, oxaliplatin, and docetaxel showed >1.8-fold increase in drug resistance, demonstrating enhanced survival capacity of resistant cell lines compared to drug-sensitive AGS cell. Rh123 intensity analysis showed reduced intracellular accumulation in AGS/5FUR, AGS/OxaR, and AGS/DoceR cells. However, verapamil treatment restored Rh123 levels in resistant cells to those of AGS cells, confirming P-gp involvement. Additionally, resistant cells adopted distinct morphological characteristics associated with acquired resistance, including irregular shapes (sickled, triangular) and increased adhesiveness, highlighting significant adaptations linked to drug resistance.

High expression of Sorcin was observed in several many drug-resistant cancers cell lines (Sung et. al., 2021; Dabaghi et. al., 2016; Gong et. al., 2014; He et. al., 2011). This study has seen that Sorcin expression in AGS cells was lower than the HEK293 cells. Also, demonstrated overexpression of Sorcin in 5FU(AGS/5FUR), oxaliplatin (AGS/OxaR), and docetaxel (AGS/DoceR) resistant GC cells. Presence of overexpressed P-gP confirmed the acquired degree of resistance in the cells against the respective drug treatments. Sorcin is highly expressed in AGS/DoceR compared to AGS/5FUR, and AGS/OxaR resistant cells. Furthermore, the drug-sensitive AGS cells, when treated with a combination of the three drugs, shown to upregulate Sorcin level. The elevation might be the combinatorial effect of the drugs or a potential combating response of the proteins to drug exposure. This observation supports the clinical observations of our study. However, the expression of Sorcin is very low in drug sensitive AGS cells unlike the cancer patients of any grades. The concentration upregulates at certain concentrations of the drugs in the cells.

More importantly, effect of docetaxel is possibly higher than 5FU and oxaliplatin in the combination therapy. It also indicates that Sorcin has developed mechanisms to counteract or neutralize the effects of the drugs and suppression of Sorcin in resistant cells indicates possible drug reversal in GC. Thus, combinatorial drug administration addresses the initial therapy success in decreasing drug-resistance, however, gradually acquire the resistance against the FLOT regimen. This implication highlights the aggressive nature of Sorcin in chemotherapy response to FLOT regimen, drug resistance and poor survival outcome in gastric cancer.

Multiple mechanisms of drug resistance involving Sorcin through major signalling pathways are employed in cancer cell lines (Li et al., 2023; Ling et al., 2021; Tong et al., 2011). Several studies have also reported the Sorcin and ERK1/2 elevation in cancer cell lines treated with different individual chemotherapeutic drugs (Wang et al., 2019; Sun et al., 2018). Herein, the clinical observations, Sorcin observed to mediate the resistance through ERK1/2 in gastric cancer. For the first time, a significant association is established between Sorcin and ERK1/2 in the overall GC population. Their

concomitant expression elevation at mRNA and protein level in non-responders and recurrent cases confers their potential interplay or regulatory mechanism in chemotherapy resistance. Even in the *in vitro* analysis the generated drug resistant AGS/5FUR, AGS/OxaR and AGS/DoceR resistance cells overexpress Sorcin and ERK1/2. Overexpression of ERK1/2 along with Sorcin and P-gP in the drug-sensitive AGS cells, after combination treatment of the three drugs, reflected the clinical observations in non-responder GC patients post-NACT. Downregulation of P-gP on Sorcin suppression signifies an interconnected signalling which is in consistent with the results of many previous studies examined in different drug-resistant cancer cell lines (Wang et al., 2019; Sun et al., 2018). Herein, the elevation of Sorcin in resistant cells and its downregulation upon siRNA transfection advocate its potential role in drug resistance. Further, attenuation of ERK1/2 level in Sorcin silenced drug-resistant cells shows regulation of these factor by Sorcin to induce drug resistance in GC. This is also illuminating the fact that the Sorcin/P-gP/ERK1/2 interaction is vitally interconnected in the signalling network imparting drug resistance mechanism. This also suggests that targeting Sorcin might impede the activation of ERK1/2 response and P-gP expression, which presents a novel therapeutic strategy for overcoming drug resistance in GC by this study.

CHAPTER 6
CONCLUSION

The salient outcomes of this study are as follows

- The study offers important insights of the demographic and clinicopathological characteristics of GC patients in Eastern India and indicators of risk stratification in an underrepresented population. The median overall survival (OS) of the cohort was 14 months [95% CI: 12.2-15.8] with a mean OS of 19.7 months [95% CI: 15.9-23.5]. Poor survival and increased hazard risk were significantly associated with older age (>60 years) [10.67 months (6.7-14.6); p<0.001; HR: 1.57(0.85-2.9)], extensive lymph node invasion, neoadjuvant FLOT chemotherapy, and chemotherapy cycles. While certain clinicopathological features like tumor size, grade, stage, and progressive weight loss, demonstrated no significant impact on survival (HE<1; p>0.05). Notably, NACT with FLOT exhibited compromised survival [17.7 months (95% CI: 11.9-23.08); p < 0.001] and significant hazard risk in non-responder undernourished patients [HR: 23.5 (6.3-88.21); p<0.001]. Conversely, a greater number of patients who undergone successful D2 lymphadenectomy and adjuvant CAPOX chemotherapy cycles associated with improved survival [24.6 months (95% CI: 16.1-33.1)]in GC patients of eastern India.

- Sorcin was significantly higher (3.17-fold) in GC tissues than in tumor adjacent (1.76-fold) with a mean difference (±SEM) of 1.41±0.505 (p=0.0077) both at transcriptional and translational level. Additionally, Sorcin level in Signet Ring Cell Carcinoma showed 5.46-fold change as compared to Tubular Adenocarcinoma tissues (4.038-fold) with a difference of 1.48±0.556 (p=0.021).

- Majority of the patients with upfront surgery demonstrated strong Sorcin expression. The mRNA level of Sorcin was evidently upregulated in NACT recipients as compared to upfront surgery cases (p=0.042). Moreover, the

level was significant in SRCC than that in TA cases (p=0.0432). On the contrary, immunostaining of Sorcin both in TA and SRCC increased in response to FLOT chemotherapy. Majority of the patients scored 3 for Sorcin immunostaining, however the patients with strong Sorcin expression were most frequent.

- Sorcin expression is significantly associated with epigastric lump, Bormann classification, tumor size, lympho-vascular invasion, Serosal invasion, pTNM stage-III, p-T3 tumor extent, and p-N2-3 lymph node status and post-treatment undernourishment both in TA and SRCC cases, ($p<0.05$).

- The patients with high Sorcin level had OS of 13 months [2.38-14.36] with log-rank (Mantle-Cox) p-value of 0.394 than those with low Sorcin expression. The univariate Cox-proportional hazard analysis showed HR of 1.32 [95% CI: 0.681-2.57] for high Sorcin expressing individuals (p=0.408). Furthermore, in TA cases, the mean OS of patients exhibiting higher Sorcin expression was 18 months [10.93-25.5; p=0.220] and HR of 0.575 [0.58-2.88; p=0.365]. The SRCC cases with higher Sorcin expression showed mean shorter OS of 10 months [6.62-15.01; p=0.065]. The univariate cox-proportional hazard analysis anticipated non-significant HR of 2.055 [0.909-4.645; p=0.084] in SRCC.

- The mRNA and protein expression of P-gP (MDR1) and ERK1/2 were assessed along with Sorcin expression. Expression of MDR1, ERK1 and ERK2 in TA and SRCC tissues with a mean of 3.63±1.35, 5.35±0.43 and 4.75±0.72, respectively. The SRCC tissues had significantly upregulated MDR1 (p=0.009), ERK1 (p=0.0003) and ERK2 (p=0.079) level as compared to TA. Furthermore, MDR1 and ERK1/2 (p=0.7194) demonstrated non-significant association with treatment response in patients post-NACT and drug resistant AGS cells (AGS/5FUR, AGS/OxaR, and AGS/DoceR). In contrary, Sorcin expression was elevated both in SRCC tissues as well as in NACT patients ($p<0.05$).

- Pearsons's correlation coefficient analysis of Sorcin with P-gP and ERK1/2 shown significant correlation among the patients who underwent NACT. Furthermore, recurrence cases showed elevated Sorcin, P-gP and ERK1/2 expression. The NACT recipients with recurrence exhibited higher Sorcin expression (2.4-fold) at protein level as compared to P-gP (1.8-fold) and ERK1 (1.7-fold).

- Protein-protein interaction result demonstrated that Sorcin-immunoprecipitated fraction showed reactivity against P-gP and ERK, indicating interactions between them.

- The effect of Sorcin silencing in the resistant cells demonstrated that suppression significantly downregulated the expression of P-gP and ERK1/2 ($p<0.05$) in AGS/5FU (90%) cells and AGS/DoceR (70%) as compared to AGS/OxaR cells.

The findings observed that poor survival associated with advanced age, extensive lymph node invasion, and neoadjuvant FLOT chemotherapy in non-responder undernourished patients, while improved survival was observed in patients receiving adjuvant CAPOX chemotherapy and D2 lymphadenectomy. The differential expression of Sorcin in GC tissues, particularly in SRCC, suggests its pivotal role in tumor progression and treatment resistance. Elevated Sorcin levels were significantly correlated with adverse clinicopathological features, including lymphovascular invasion, advanced tumor stage, and post-treatment undernourishment, underscoring its prognostic value. Importantly, Sorcin demonstrated significant interactions with P-gP and ERK1/2, with higher expression observed in SRCC and NACT recipients. Sorcin's interaction with P-gP and ERK, implicating its role in chemoresistance through this axis. Furthermore, Sorcin silencing in resistant cell lines led to significant downregulation of P-gP and ERK1/2 expression, highlighting its potential as a therapeutic target in overcoming drug resistance.

Future research will focus on developing Sorcin-targeted therapies, and validating its role as a biomarker for predicting chemotherapy response and prognosis in a larger cohort.

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ANNEXURE I

INSTITUTIONAL ETHICS COMMITTEE

Chittaranjan National Cancer Institute
An autonomous body under the Ministry of
Health & Family Welfare, Govt. Of India
37, S.P.Mukherjee Road, Kolkata-700026, WB, India
Tel: 2475-9313/8057, Fax: +91-33-2475-7606
Web: www.cnci.org.in

IEC Ref: CNCI-IEC-32846-2020 Date: 04.12.2020

Dr. Vilas Nasare, CNCI

Sub.: IEC decision on review of the project submitted for approval.

Protocol Title: **Study on Sorcin mediated pathway of Multi Drug Resistance in Gastric Carcinoma**

Study Site: Chittaranjan National Cancer Institute, Kolkata

The Chairman and members of Institutional Ethics Committee (IEC) reviewed the project and other related documents submitted by you for the proposed study entitled "**Study on Sorcin mediated pathway of Multi Drug Resistance in Gastric Carcinoma.**"

The following documents were reviewed:

1. Model form to be filled by the Principal Investigator (PI) for submission to Institutional Ethics Committee (IEC)
2. Project with Summary
3. Informed Consent Sheets
4. Administrative Approval
5. Approval from Academic Committee

The committee should be informed:

I. About the progress of the study annually
II. Any changes in the protocol and patient information/ informed consent documents, prior to their implementation

The Project is approved by IEC.

Final report of the study shall have to be submitted to the IEC in all cases, even when the study is abandoned for any reason(s).

Yours Sincerely,

Member Secretary
IEC, CNCI
7/12/2020

Member Secretary
Institutional Ethics Committee
Chittaranjan National Cancer Institute
37, S. P. Mukherjee Road, Kokata-700026

Chairman
IEC, CNCI
10/12/2020

Chairman
Institutional Ethics Committee
Chittaranjan National Cancer Institute
37, S. P. Mukherjee Road, Kokata-700026

ANNEXURE II

Chittaranjan National Cancer Institute

37, S.P. Mukherjee Road, Kolkata -700026

Informed Consent Form

Project Title: Study on Sorcin Mediated Pathway of Multidrug Resistance in Gastric Carcinoma

Name of the participant/Patient: **Registration No.:**

Documentation of the Informed Consent:

I, _____, have read and understood the information provided in this form (or it has been explained to me). I was free to ask any questions, and they have all been answered to my satisfaction. I confirm that I am 18 years of age or older and, exercising my free will, hereby consent to participate in the project entitled 'Study on Sorcin Mediated Pathway of Multidrug Resistance in Gastric Carcinoma'

i. I have read and understood this consent form and the information provided to me.
ii. I have had the consent document explained to me.
iii. I have been explained the nature of the study.
iv. My rights and responsibilities have been explained to me by the investigator.
v. I have informed the investigator of all the treatments I am currently taking or have been taking for the past ___ months which also includes any 'desi' (alternative) treatments.
vi. I hereby give permission to the investigator to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Government agencies and ethics committee. I understand that they may inspect my original records.
vii. My identity will be kept confidential if my data are publicly presented.
viii. I have had my questions answered to my satisfaction.
ix. I have decided to part in the research study.

I am aware that if I have any questions during the study, I should contact the Mentor/ Principal Investigators/ Co- investigators of the project. By signing this consent form, I attest the information in this document. I will be given a copy of this consent document.

_____

Participant's (or legal representative if participant is incompetent) signature/ Thumb impression

_____

Name and Signature of impartial witness (required for illiterate patients)

Date: Time: Place:

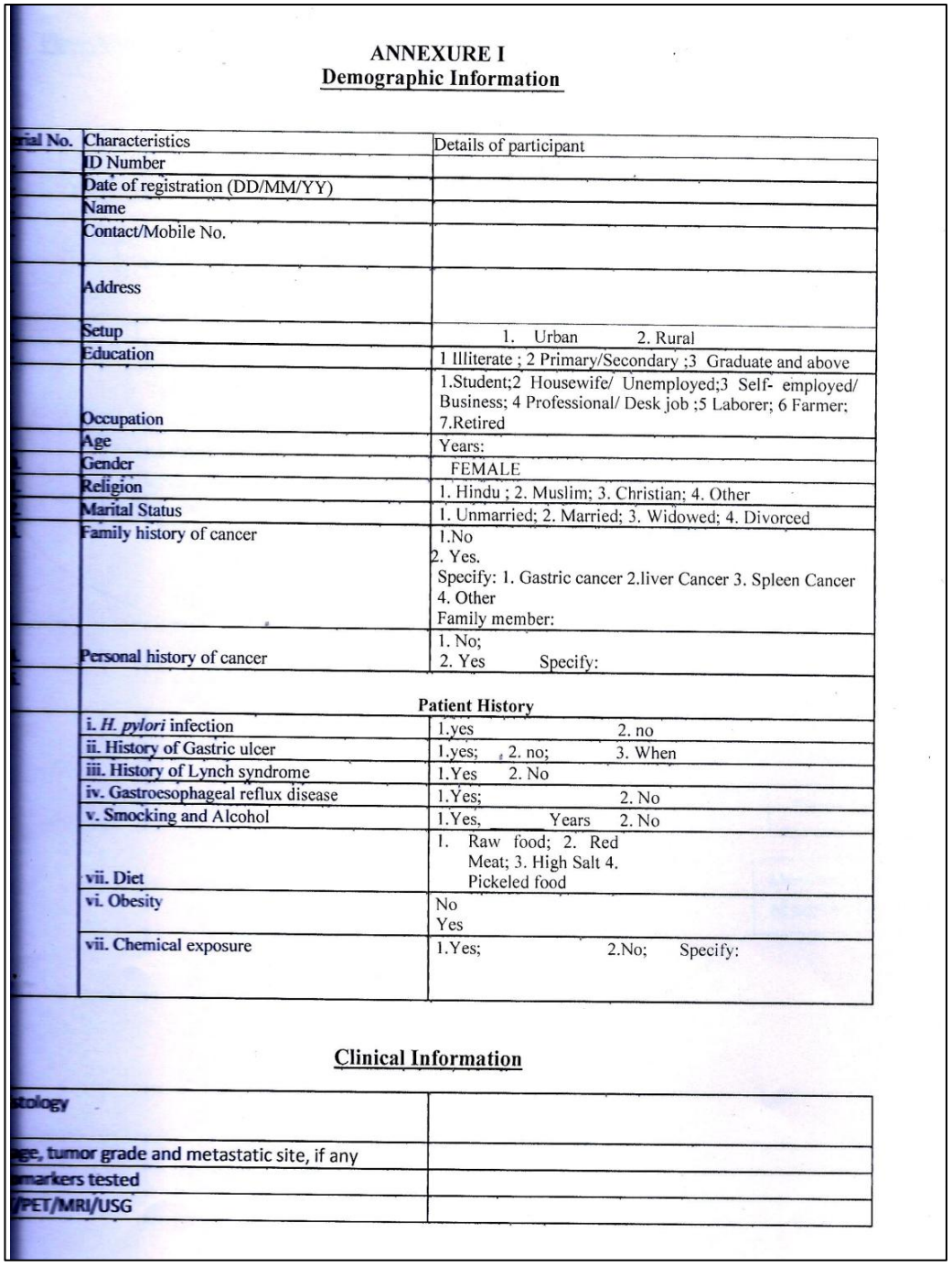
Address and Contact number of impartial witness:

_____

Name and signature of the Mentor/PI/ Co-PI:

Name: Signature: Date:

ANNEXURE III



ANNEXURE I
Demographic Information

rial No.	Characteristics	Details of participant
	ID Number	
	Date of registration (DD/MM/YY)	
	Name	
	Contact/Mobile No.	
	Address	
	Setup	1. Urban 2. Rural
	Education	1 Illiterate ; 2 Primary/Secondary ;3 Graduate and above
	Occupation	1.Student;2 Housewife/ Unemployed;3 Self- employed/ Business; 4 Professional/ Desk job ;5 Laborer; 6 Farmer; 7.Retired
	Age	Years:
	Gender	FEMALE
	Religion	1. Hindu ; 2. Muslim; 3. Christian; 4. Other
	Marital Status	1. Unmarried; 2. Married; 3. Widowed; 4. Divorced
	Family history of cancer	1.No 2. Yes. Specify: 1. Gastric cancer 2.liver Cancer 3. Spleen Cancer 4. Other Family member:
	Personal history of cancer	1. No; 2. Yes Specify:
	Patient History	
	i. *H. pylori* infection	1.yes 2. no
	ii. History of Gastric ulcer	1.yes; 2. no; 3. When
	iii. History of Lynch syndrome	1.Yes 2. No
	iv. Gastroesophageal reflux disease	1.Yes; 2. No
	v. Smocking and Alcohol	1.Yes, Years 2. No
	vii. Diet	1. Raw food; 2. Red Meat; 3. High Salt 4. Pickeled food
	vi. Obesity	No Yes
	vii. Chemical exposure	1.Yes; 2.No; Specify:

Clinical Information

stology	
ge, tumor grade and metastatic site, if any	
markers tested	
/PET/MRI/USG	

ANNEXTURE IV

Sl. no.	Product Name	Catalogue Number	Manufacturer
1	IHC kit	DAB150	ABclonal
2	Xylene	AS080	Himedia
3	Hematoxylin	H9627	Sigma-aldrich
Cell lines and cell culture chemicals			
4	Ham's F-12 K nutrient media	AT025	HiMedia
5	DMEM nutrient media	AT195	
6	Sodium Pyruvate	PCT0503	
7	Sodium bicarbonate	TC230	
8	FBS (heat inactivated; south Africa origin)	RM 9955	
9	penicillin/streptomycin	A028	
10	Trypsin-EDTA	TCL034	
11	MTT	MB186	
12	DMSO	MB058	
13	Opti-MEM w/ HEPES	31985-070	Gibco
14	Rhodamine 123	83702	Sigma-Aldrich
15	siRNA control	Sc-37007	Santa Cruz
16	Sorcin siRNA	SC-41016	
17	Lipofectamine 2000	1168-027	Invitrogen
Primary antibodies			
18	Sorcin	A6751	ABclonal
19	P-gP	A11758	
20	p-ERK1/2	Kit#9911	Cell Signaling Technology
21	Sorcin	sc-100859	Santa Cruz
22	Ras	C8-166221	
23	Raf	E-10-sc7667	
24	MEK	Sc-6250	
25	ERK1	Sc-27169	
26	ERK2	Sc-1647	
27	β-actin	ab8224	abcam

Secondary antibodies			
28	HRP Goat Anti-rabbit IgG	AS014	ABclonal
Western blot kit			
29	ECL	RM0286	Taurus
30	BSA	TC546	HiMedia
31	PVDF membrane	IPVH00010	Merk
32	TEMED	K49975532 807	Merk
33	Ammonium persulphate (APS)	GRM1094	Himedia
34	Bradford Reagent	ML106	
35	PIC	11836170001	Sigma-aldrich
36	PMSF	TC706	Himedia
37	NaF	GRM1081	
qRT-PCR kit			
38	mRNA primer		IDT
39	TI script II cDNA first strand synthesis kit	TIMB20400L	Taurus
40	Taq 2X PCR master mix with dye	TIMB20604L	
41	SYBER green	TIMB21204SS	
42	TRIzol reagent	301-001	
43	Taq DNA polymerase (Mg2+ plus buffer)	TIMB20600	
44	Chloroform	MB109	Himedia
Co-immunoprecipitation kit			
45	Protein A Agarose beads	16-125	Merk

JADAVPUR UNIVERSITY

Kolkata- 700032, India

Index Number. 116/21/Life Sc./27

1. Title of the thesis: Study on Sorcin Mediated Pathway of Multidrug Resistance in Gastric Carcinoma

2. Name, Designation, and Institute of the Supervisor(s):

Dr. Vilas D. Nasare

Senior Scientific Officer
Department of Pathology and Cancer Screening
Chittaranjan National Cancer Institute
37, S.P. Mukherjee Road, Kolkata-700 026
India

3. List of Publication

From thesis

Published

i. **Ghosh S**, Banerjee R, Chakrabarti J, Alam N, Nath P, Mukherjee KK, D Nasare VD. Impact of Clinicopathological Factors and Treatment Outcomes on Gastric Cancer Survival: A Tertiary Care Hospital based Study in Eastern India. **Journal of Cancer Research and Therapeutics**, 2025. DOI: 10.4103/jcrt.jcrt_655_24.

ii. **Ghosh S**, et al. Comparative study of ERK1/2, Bcl2, and Sorcin with Clinicopathological Characteristics of Gastric carcinoma: Tubular Adenocarcinoma Vs Signet Ring Cell Carcinoma. **APJC** 2025. (accepted Feb 25)

iii. **Ghosh S**, Sharma A, Kumar RS, Nasare V. Sorcin: mechanisms of action in cancer hallmarks, drug resistance and opportunities in therapeutics. **Medical Oncology** 2024 Dec 14;42(1):29. doi: 10.1007/s12032-024-02580-6.

Research Papers Under peer review

i. **Ghosh S**, et al. Sorcin Overexpression in Chemoresistance of FLOT-treated Gastric Carcinoma Patients. American Journal of Clinical Oncology.

Other publications

i. Mistry T, Pal R, **Ghosh S**, Choudhury T, Mandal S, Nath P, Alam N, Nasare VD. Impact of Low BMI and Nutritional Status on Quality of Life and Disease Outcome in Breast Cancer Patients: Insights from a Tertiary Cancer Centre in India. Nutrition and Cancer 2024, doi.org/10.1080/01635581.2024.2347396.

ii. Sahoo, P. K., Mistry, T., **Ghosh, S**., Pal, R., Mahata, S., Sarkar, S., Nasare, V. D. (2024). Polymorphism of Transporter Genes Contributes to Variations in Treatment Outcomes and Adverse Events in Platinum Based- Chemotherapy-Treated Oral Cancer Patients. *Precision* ***Oncogenomics***, *1*(1). https://doi.org/10.1080/28354311.2023.2292798.

iii. Kumar, S., Bishnoi, P., Kumar, G., Saroha, B., Kumari, B., Lathwal, E., **Ghosh, S,** Nasare, V. D. (2025). Unlocking the Anticancer potential of 6-propargyloxyaurone against AGS cancer cell line: design, synthesis, SAR, DFT, Hirshfeld surface and molecular docking analysis. **Journal of Molecular Structure**, *1327*, 141213.

iv. Kumar, G., Saroha, B., Arya, P., Ghosh, S., Kumari, B., **Ghosh, S,** Nassare, V. D., ... & Kumar, S. (2025). 1, 2, 3-triazole clubbed and dichloro substituted novel aurones as potential anticancer agents targeting digestive enzymes: design, synthesis, DFT, ADME and molecular docking studies. *Journal of Molecular Structure*, *1319*, 139460.

v. Mahata, S., Behera, S., Kumar, S., **Ghosh, S**., Nasare, V. D. (2024). PIM1-STAT3 mediated docetaxel induced epithelial-mesenchymal transition related to in silicoheterodimeric STAT3-PIM1-Docetaxel complex in breast cancer cells (In press).

vi. Kumar G., Saroha B., Kumari B., **Ghosh S.,** Nasare VD., Kumar S. Exploring the Antiproliferative Potential of Morpholine-Functionalized Aurones: Design, Synthesis, SAR, DFT, Hirshfeld Surface, 3D Energy Frameworks and Molecular Docking Analysis. **Chemistry Select** 2024 May 22;9 (20): e202400749. https://doi.org/10.1002/slct.202400749.

vii. Lathwal E, Kumar S, Sahoo PK, **Ghosh S,** Mahata S, Nasare VD, Kapavarapu R, Kumar S. Pyrazole-based and N, N-diethylcarbamate functionalized some novel aurone analogs: Design, synthesis, cytotoxic evaluation, docking and SAR studies, against AGS cancer cell line. **Heliyon**. 2024 Feb 24;10(5): e26843. doi: 10.1016/j.heliyon. 2024.e26843.

viii. Sahoo PK, Bhowmick AK, Sarkar S, Mahata S, Pal R, Mistry T, **Ghosh S**, Choudhury T, Kumar RS; Mondal S, Datta S, Nath P, Mukherjee KK, Nasare VD. Outcomes of 3-year follow up with induction vs first line chemotherapy in oral cancer patients: An observational hospital-based study. **Journal of Cancer Research and Therapeutics**: January 22, 2024. | DOI: 10.4103/jcrt.jcrt_2179_22.

ix. Mistry T, Nath A, Pal R, **Ghosh S**, Mahata S, Kumar Sahoo P, Sarkar S, Choudhury T, Nath P, Alam N, Nasare VD. Emerging Futuristic Targeted Therapeutics: A Comprising Study Towards a New Era for the Management

of TNBC. **Am J Clin Oncol**. 2024 Mar 1;47(3):132-148. doi: 10.1097/COC.0000000000001071.

x. Mahata S, Sahoo PK, Pal R, Sarkar S, Mistry T, **Ghosh S**, Nasare VD. PIM1/STAT3 axis: a potential co-targeted therapeutic approach in triple-negative breast cancer. **Med Oncol**. 2022 May 15;39(5):74. doi: 10.1007/s12032-022-01675-2.

xi. Lathwal, E., Kumar, S., Sahoo, P. K., **Ghosh, S.**, Mahata, S., Nasare, V. D., & Kumar, S. (2022). Synthesis, cytotoxic evaluation and structure activity relationship of pyrazole hybrid aurones on gastric cancer (AGS) cell lines. **Results in Chemistry**, 4, 100590.

xii. Saroha B, Kumar G, Kumar S, Kumari M, Rani M, Raghav N, Sahoo PK, **Ghosh S**, Mahata S, Nasare VD. Novel 1, 2, 3-triazole-aurone hybrids as cathepsin B inhibitors: One-pot synthesis, anti-proliferative, and drug modeling studies. **European Journal of Medicinal Chemistry Reports**. 2022 Aug 1; 5:100056.

xiii. Saroha B, Kumar G, Kumar S, Kumari M, Rani M, Raghav N, Sahoo PK, **Ghosh S**, Mahata S, Nasare VD. Ultrasound assisted a one pot multicomponent and greener synthesis of 1, 2, 3-triazole incorporated aurone hybrids: Cathepsin B inhibition, anti-cancer activity against AGS cell line, and in-silico docking evaluation. **Current Research in Green and Sustainable Chemistry**. 2022 Jan 1; 5:100295.

xiv. Sarkar S, Pal R, Mahata S, Sahoo PK, **Ghosh S**, Chatterjee P, Vernekar M, Mandal S, Bera T, Nasare VD. Evaluation of numerical rating scale and neuropathic pain symptom inventory pain scores in advanced ovarian carcinoma patients undergoing surgery and first-line chemotherapy. **J Clin Transl Res**. 2022 Jan 25;8(1):54-60.

xv. Sarkar S, Sahoo PK, Mahata S, Pal R, Ghosh D, Mistry T, **Ghosh S**, Bera T, Nasare VD. Mitotic checkpoint defects: en route to cancer and drug resistance. **Chromosome Res**. 2021 Jun;29(2):131-144. doi: 10.1007/s10577-020-09646-x.

xvi. Sahoo PK, Sarkar S, Ghosh D, Mahata S, Pal R, Mistry T, **Ghosh S**, Roy A, Bucha H, Mandal S, Nasare VD. Premalignant and malignant lesions of oral cavity in eastern India: a hospital-based study. **Eur J Cancer Prev**. 2021 Sep 1;30(5):393-399. doi: 10.1097/CEJ.0000000000000640. PMID: 33252366.

4. List of Patents: None

5. List of Presentations in National/International/Conferences/Workshops:

From thesis

i. 75th Academic Awards and Medals 2023-2024. Organized by The Zoological Society of Kolkata, October 2023 – Oral Presentation. Awarded ZSK Special Award for Scholars (2023). **Sushmita Ghosh**, Tanuma Mistry, Arpana Sharma, Ranita Pa[1], Trisha Choudhury, Jayanta Chakrabarti, Neyaz Alam, Kalyan Kusum Mukherjee, Vilas D Nasare. Interplay of Sorcin and ERK1/2 Expression in Gastric Cancer Patients Undergoing Neoadjuvant Chemotherapy.

ii. 25th ESMO World Congress on Gastrointestinal Cancer (2023, Barcelona, Spain) – Poster Presentation (Abstract published). **Ghosh S**, et al. P-5: Sorcin in Multidrug-Resistant Gastric Cancer Patients. **Annals of Oncology**, 2023.

iii. American Society of Clinical Oncology (ASCO) Annual Meeting (2023, Chicago, USA) – (Abstract Published). **Ghosh S**, et al. ERK1/2 Overexpression and Chemoresistance in Advanced Gastric Cancer. **Journal of Clinical Oncology**, 2023.

iv. INCD2022 5th International Conference on Nutraceuticals for Cancer and Other Chronic Disease. Organized by Department of Zoology, University of Delhi, Delhi, October 7-9,2022, OP33, Page 111.-Oral presentation. **Sushmita Ghosh**, Tanuma Mistry, Ranita Pal, Pranab Kumar Sahoo, Sutapa Mahata, Sinjini Sarkar**,** Trisha Choudhury, Raya Banerjee, Jayanta Chakrabarti, Kalyan Kusum Mukherjee, Neyaz Alam, Shyamsundar Mandal, Vilas D Nasare Role of Sorcin in the development of multidrug resistance against 5-Flurouracil, Leucovorin, oxaliplatin and Docetaxel (FLOT) chemotherapeutic regimens in the Gastric cancer subtypes.

Other publications

i. Mistry, T., **Ghosh, S**., Sahoo, P., Mahata, S., Pal, R., Sarkar, S., ... & Nasare, V. D. (2022). Single nucleotide polymorphisms of ABCB1 (rs1128503) and ABCC2 (rs145008610) genes and its clinical impact in ER & PR positive breast cancer patients in a tertiary care hospital of India. European Journal of Cancer, 175, S79. https://doi.org/10.1016/S0959-8049(22)01565-9

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Original Article

Impact of clinicopathological factors and treatment outcomes on gastric cancer survival: A tertiary care hospital-based study in Eastern India

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ABSTRACT

Objective: The late diagnosis of GC poses a significant health burden worldwide, particularly in Asia. Despite the declining incidence, the heterogeneous diversity of India exhibits pronounced regional disparity in survival rates among the patients. The study observed the clinicopathological factors and treatment outcomes influencing the survival of gastric cancer (GC) patients.

Methods: A total of 78 newly diagnosed GC patients were enrolled (from 2019 to 2023). Overall survival (OS) and hazard risk (HR) for sociodemographic, clinicopathological factors, and treatment outcomes of the patients were assessed by univariate Kaplan-Meier and multivariate Cox-Regression analysis.

Results: The frequency of GC was highly prevalent among males (69.2%), with a median age of 52 ± 11.35 years. The majority of them are associated with mixed diet (88.5%), grade-III tumors (57.7%) located in the antrum (60.3%) at ≥5 cm (56.4%) classified as pTNM stage-III (64.1%) exhibiting lymphovascular invasion (75.6%). Clinical features, including initial symptoms, treatment response, and pTNM-stage presented increased hazard risks in the patients (HR >1) but presented no significant difference. Nearly 63% of the patients operated upfront and 37% received neoadjuvant FLOT chemotherapy. The mean overall survival of the patients was 19.7 months [95% CI: 15.9–23.5]. The hazard of death was significantly allied with older age ($P < 0.001$), and neoadjuvant–FLOT–chemotherapy ($P < 0.001$); however, a higher number of cycles of adjuvant–CAPOX–chemotherapy associated with improved overall survival ($P < 0.001$) and relatively lower HR.

Conclusion: The clinicopathological attributes and treatment outcomes like older age, weight loss, tumor size, type of gastrectomy, FLOT regimen, number of cycles, and postoperative undernourishment demonstrated higher HR and compromised survival in GC patients of Eastern India.

KEY WORDS: Chemotherapy, clinicopathology, gastric cancer, overall survival, upfront surgery

INTRODUCTION

Gastric cancer (GC) is the 5th most common cancer globally, and its prevalence is restricted to certain regions of the world. The highest incidence of GC was reported in Asia, particularly in China and Japan. Contrastingly, India holds the 7th position, experiencing a decrease in GC cases compared to China, Japan, and Korea.[1] The observed net 5-year survival of India is relatively poor, standing at 8.3 (20–40%), indicating poor prognosis among the patients.[1,2] Despite the decline, the mortality rate remains comparably higher in younger Indian men, positioning GC as the third leading cause of cancer-related deaths in this demographic.[3,4] The trend is a notable concern in southern urban registries but is on the rise in rural northern states. These significant regional disparities are due to burdens like an aging population, late-stage diagnosis, limited access to treatment, and high risks of recurrence in India.[5,6]

In a significant proportion of the Indian population, dietary habits, smoking, tobacco use, and *H. pylori*

Cite this article as: Ghosh S, Banerjee R, Chakrabarti J, Alam N, Nath P, Mukherjee KK, *et al.* Impact of clinicopathological factors and treatment outcomes on gastric cancer survival: A tertiary care hospital-based study in eastern India. J Can Res Ther 2025;XX:XX-XX.

Submitted: 02-Apr-2024
Revised: 20-Sep-2024
Accepted: 19-Dec-2024
Published: ***

Access this article online
Website: https://journals.lww.com/cancerjournal
DOI: 10.4103/jcrt.jcrt_655_24
Quick Response Code:

infection are key etiological factors that increase the risk of GC, and contribute to a compromised survival rate.[7] Additionally, the family history of cancer and the history of an individual relay a higher genetic susceptibility to the disease. However, the lack of awareness, screening program, asymptomatic nature of the disease, and communal presenting features hinder early detection leading to tumor aggressiveness encompassing advanced staging, higher grade, and metastasis. These attributes act as an independent prognostic indicator, prompting the hazard risk of death in GC patients.[8,9]

Over the past decade, neoadjuvant chemotherapy has emerged as the standard treatment for locally advanced GC compared to upfront surgery and chemoradiotherapy.[10] Even in India, the perioperative or neoadjuvant chemotherapy comprising 5-fluorouracil, leucovorin, oxaliplatin, and docetaxel (FLOT) regimen noted a fairly higher 5-year survival advantage surpassing upfront surgery in patients having good ECOG performance status.[11,12] However, the outcome of multimodal treatment approaches varies among patients due to diverse clinical and pathological features across different regions of India.

The study aims to comprehend the impact of heterogenous sociodemographic, clinicopathological features, and treatment outcomes on the survival of GC patients in West Bengal, situated in Eastern India.

MATERIALS AND METHODS

Study design and patient recruitment

Initially, 103 patients were selected, but only 78 were able to complete their follow-ups, while the remaining 25 patients were either defaulters or lost to follow-up. A total of 78 patients diagnosed with GC between December 2019 and 2023 were included in the study, as outlined in the study design [Figure 1]. This prospective study was carried out with the help of the Departments of Surgical Oncology, Chemotherapy, and Pathology at Chittaranjan National Cancer Institute (CNCI), Kolkata, India. The patients were selected based on specific inclusion and exclusion criteria. The eligible patients were those aged ≥18 years and older, diagnosed with stage I–III gastric adenocarcinoma, who had not yet received chemotherapy and exhibited adequate bone marrow, platelet count, hepatic, and neurologic function. Patients must be eligible for oral administration and be willing to comply with scheduled visits and treatment plans. Before surgery, patients may receive FLOT chemotherapy as a first-line treatment. Exclusion criteria included patients under 18 years with distant metastases, GI obstruction, prior radiotherapy/chemotherapy, and certain medical conditions. Pregnant or breastfeeding women, individuals at risk of pregnancy, and those with specific complications, allergies, or unwillingness to participate are also excluded. Ethical approval was obtained, and informed consent was signed by participants or their guardians in English, Hindi, or Bengali.

Data collection

Demographic and clinicopathological information were acquired from patient files. The demographic data at diagnosis included age, gender, religion, habitation, marital status, occupation, income, diet, initial symptoms, addiction history, family or personal history, treatment recommended, surgical type, ECOG performance status, and history of chemotherapy. The core dataset of pathological characteristics collected after surgery included primary tumor location, tumor size, tumor gross type, pathological grading, the extent of lymphovascular invasion (LVI), perineural invasion (PNI), and serosal invasion (SI), radiological findings, recurrence, survival status, postoperative complications (intra-abdominal hemorrhage, wound infection, anastomotic leakage, abdominal infection, and other comorbidities), and nutritional status.

Treatment

Forty-nine participants were eligible for upfront surgery (D2 lymphadenectomy), followed by adjuvant chemotherapy (ACT) with Oxaliplatin (130 mg/m^2) and Capecitabine (1000 mg/m^2/oral twice daily, repeated for 21 days). Twenty-nine patients received neo-adjuvant chemotherapy (NACT) followed by surgery (D2 lymphadenectomy). The NACT patients were administered with 4 cycles of a FLOT regimen composed of a 2-hour infusion of 5-Fluouracil (500–2500 mg/m^2) with Leucovorin (200 mg/m^2), Oxaliplatin (85 mg/m^2), and docetaxel (60 mg/m^2) as first-line chemotherapy. Treatment was repeated every 2 weeks until disease progression, patient denial, or intolerable adverse responses.[13]

Evaluation of clinical response after chemotherapy

The post-chemotherapy clinical response was assessed after each cycle using a Contrast-Enhanced Computed Tomography (CECT) scan, thorax imaging, and PET-CT scan. Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST, v1.1) was used as a standardized method to measure lesions to determine whether a tumor has disappeared, shrunk, remained unchanged, or increased in size, thus categorizing responses, and assessing tumor progression. Patients who responded to chemotherapy were classified as complete (CRs) or partial (PRs) responders, while patients with stable disease, or progressive disease were considered non-responders (NRs).[14] The overall response rate (ORR) was calculated based on the number of CRs and PRs after chemotherapy. Following these radiological confirmations, surgery was performed, and chemotherapy cycles were continued until drug intolerance and disease progression occurred.

Assessment of overall survival (OS) and Hazard risk (HR)

Oncological outcomes included OS which was calculated as the time interval from the date of diagnosis to death or the last date of follow-up. The survival month was noted until patients either succumbed to death, were censored (survived), or lost contact during the observation period. Cox regression (cox proportional hazard ratio) was applied to evaluate the effects of the risk factors among GC patients.

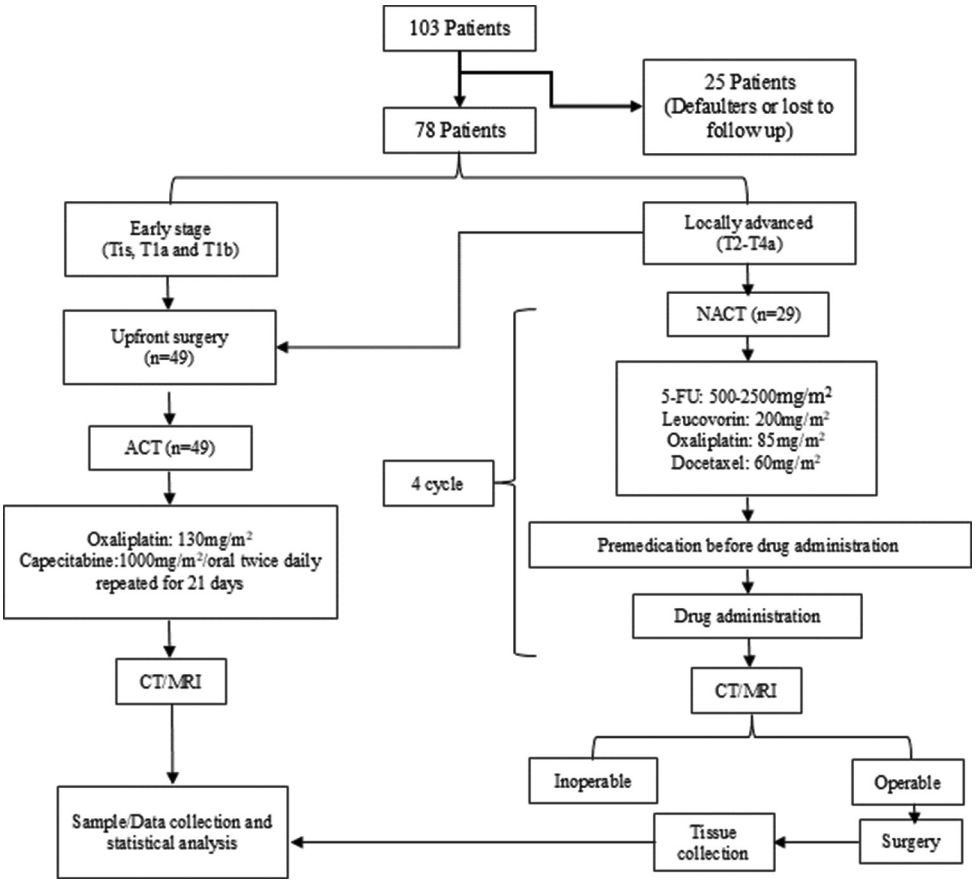

Figure 1: Flowchart representing Study design of locally advanced gastric cancer patients

Statistical analysis

All analyses were performed in IBM SPSS V.27.0.1 software, with a two-sided *P* value of less than 0.05 considered statistically significant. Descriptive statistics were applied to categorical data related to patient demographics and clinical characteristics, presented as mean ± SD, frequency, and percentage. Comparisons of variables were evaluated using the Chi-Square test for the categorical data (cross-tabulation). Kaplan–Meier univariate analysis was performed to estimate the mean survival month for each clinical parameter, with comparisons made using the log-rank test, significant at $P < 0.05$. The multivariable Cox regression model assumed proportional hazard risk for all GC parameters, with associations reported as hazard ratios (HR) and 95% confidence intervals (CIs).

RESULTS

Sociodemographic and clinicopathological characteristics of GC patients

After screening 78 patients, there were 54 males and 24 females, diagnosed at a median age of 52 ± 11.35 years (range 28–79 years), with 58 (74.4%) residing in rural areas of Eastern India. The majority were married (47, 60.2%), spoke Bengali (71, 91.0%), identified as Hindus (61, 78.2%), and followed a mixed diet (69, 88.5%). Primarily they were laborers (41, 52.5%), and 42 (53.8%) reported earning between 2001 and 10000 rupees. Most patients reported no addictions (25, 32.1%), while 22 (28.2%) admitted to admitted to using a combination of smoking, alcohol, and tobacco. Additionally, 8 patients (10.2%) reported a family history of chronic gastric issues or malignancy, 47 (60.3%) had a personal history of peptic disorders, and 47 (60.3%) exhibited wall thickening, with an ECOG score of 1 [Table 1].

Survival analysis of GC patients with different clinical, pathological characteristics and treatment outcomes

Overall survival and clinical features of GC patients

Fifty-two patients (66.7%) succumbed to death, while 26 patients (33.3%) were censored. The median overall survival (OS) of the cohort was 14 months [95% confidence interval (CI): 12.2–15.8] with a mean OS of 19.7 months [95% CI: 15.9–23.5] [Figure 2]. Forty-seven (60.3%) patients aged between 41 and 60 years had a significantly lower mean OS of 10.67 months [95% CI: 6.7–14.6] ($P < 0.001$) with a significant hazard ratio (HR) of 2.003 [95% CI: 1.2–2.3] ($P = 0.002$). Males had an HR of 1.57 [95% CI: 0.85–2.9], indicating a higher risk of death compared to females. The antrum was the most prevalent tumor site, accounting for 55 patients (70.5%), with an OS of 17.7 months [95% CI: 13.8–21.7]. However, the HR of 0.977 [95% CI: 0.83–1.3] suggested no significant difference in OS based on the tumor site ($P = 0.769$). Among the cohort,

Table 1: Cumulative frequency (%) of demographic, and clinicopathological characteristics of gastric cancer patients

Characteristics (*N*=78)		Frequency (*n* (%))
Age (Years)	19–40	14 (17.9)
	41–60	47 (60.3)
	>60	20 (21.8)
Gender	Male	54 (69.2)
	Female	24 (30.8)
Habitation	Rural	58 (74.4)
	Urban	20 (25.6)
Religion	Hindu	61 (78.2)
	Muslim	17 (21.8)
Language	Bengali	71 (91.0)
	Hindi	7 (9.0)
Occupation	Labor	41 (52.5)
	Government or Private farm employee	22 (28.2)
	Unemployed	15 (19.2)
Income	<2000 Rs	1 (1.3)
	2001–10000 Rs	42 (53.8)
	10001–20000 Rs	29 (37.2)
	<20000 Rs	6 (7.7)
Marital status	Unmarried	14 (17.9)
	Married	47 (60.2)
	Widowed	17 (21.7)
Diet	Mixed diet	69 (88.5)
	vegetarian	9 (11.5)
Addiction history	Nil	25 (32.1)
	Smoking	21 (26.9)
	Alcohol	6 (7.7)
	Tobacco chewing	4 (5.1)
	Smoking, alcohol and tobacco	22 (28.2)
Family history	No history	70 (89.7)
	Gastric ulcer	3 (3.8)
	Gastritis	3 (3.8)
	Malignancy	2 (2.6)
Past history of the patient	Nil	30 (38.5)
	Peptic disorder	47 (60.3)
	Carcinoma larynx, chronic gastritis	1 (1.3)
Tumor type	Adenocarcinoma	77 (98.7)
	Gastrointestinal stromal tumor (GIST)	1 (1.3)
Radiological findings	Ulceration	12 (15.4)
	Growth	16 (20.5)
	Wall thickening	47 (60.3)
	Wall thickening and ulceration	2 (2.6)
	Patchy erythema	1 (1.3)
Eastern cooperative oncology group (ECOG) performance status	0 (asymptomatic, fully active)	19 (24.4)
	1 (completely ambulatory, restricted to physically strenuous activities)	47 (60.3)
	2 (50% in bed but capable of self-care)	7 (9.0)
	3 (50% confined to bed and capable of limited self-care)	4 (5.1
	4 (bedbound, completely disabled)	1 (1.3))

N=total number of individuals; *n*=number of individuals; %=percentage

44a (56.4%) had a tumor size of ≥5 cm, while 34 (43.6%) had a tumor size of ≤5 cm at diagnosis. Patients with tumor size ≤5 cm had a lower mean OS of 15.6 months [95% CI: 11.5–19.4], compared to those with tumor size ≥5 cm, who had an OS of 22.5 months [95% CI: 16.5–28.3], resulting in an HR of 1.3 [95% CI: 0.4–1.3] [Table 2].

Overall survival and histopathological characteristics of GC patients

The histopathology analysis categorized the grade and various classifications of GC. According to the Lauren classification, 45 patients (57.7%) had the intestinal type, and 33 patients (42.3%) had the diffuse type GC; however, depicted no significant association in OS within these groups ($P > 0.05$). The WHO classification identified 37 patients (47.4%) with tubular adenocarcinoma and 32 patients (41.0%) with signet-ring cell carcinoma. Mucinous adenocarcinoma was observed in 6 patients (7.7%), with the lowest mean OS of 9.5 months [95% CI: 4.61–14.38]. The mean OS for signet-ring cell carcinoma was 16.6 months [95% CI: 12.1–21.1], with no significant hazard risk (HR <1). The lympho-vascular invasion (LVI) was significant in 59 patients (75.6%), while perineural invasion (PNI) was noted in 23 patients (29.5%), and serosal invasion was present in 15 patients (19.2%). The HR for PNI and serosal invasion was 1.17 [95% CI: 0.64–2.1] and 1.42 [95% CI: 0.69–2.9], respectively, indicating no significant difference in mean OS within these groups. The grade III patients exhibited a lower mean OS of 17.2 months [95% CI: 12.9–21.5], whereas, grade I patients had the highest mean OS of 19.5 months [95% CI: 2.2–36.7] [Table 2]. Grade differentiation included 4 patients (5.1%) with Grade 1 (well differentiated), 29 patients (37.2%) with Grade II (moderately differentiated), and 45 patients (57.7%) with Grade III (poorly differentiated) adenocarcinoma. The χ^2-test analyzed a significant correlation between grade differentiation and age ($P = 0.009$), gender ($P = 0.03$), epigastric lump ($P = 0.028$), ≥5 cm tumor size ($P < 0.001$), wall thickening ($P = 0.002$), Type III Borrmann classification ($P < 0.001$), diffuse type ($P < 0.001$), signet ring cell carcinoma ($P < 0.001$), LVI ($P < 0.001$), *p*T3 tumor extent ($P < 0.001$), *p*N2 and *p*N3a lymph node invasion ($P < 0.001$), *p*TNM stage-IIIA ($P = 0.001$), and postoperative undernourishment ($P = 0.041$) [Figure 3].

Overall survival and initial symptoms of GC patients

A total of 72 patients (97.4%) reported abdominal pain and 69 patients (89.7%) experienced progressive weight loss. Anorexia was observed in 62 patients (42.3%), while dysphagia was less common, affecting only 12 patients (8.9%). Nausea, vomiting, and dyspepsia were reported by over 70% of the individuals. These symptoms are frequently associated with gastric issues, which aligns with the history of peptic disorders noted in 42 patients (53.8%). Another common presenting feature of advanced GC was the epigastric lump, experienced by 44 patients (55.1%), while anemia was observed in 48 patients (67.9%). Additionally, constipation and melena were reported by 60 patients (76.9. 1%) (data not shown). The mean OS for patients experiencing weight loss was significantly lower, at 18.3 months [95% CI: 14.2–22.5] ($P = 0.05$) with a significant hazard ratio (HR) of 2.84 [95% CI: 1.01–7.99] ($P = 0.038$). Moreover, except for constipation, other presenting symptoms indicated higher hazard risk but remained non-significant (HR > 1) [Figure 4].

Overall survival and treatment response of the GC patients

Forty-nine patients (62.8%) operated upfront followed by adjuvant chemotherapy, while 29 patients (37.2%) received NACT before surgery. Surgical procedures included total,

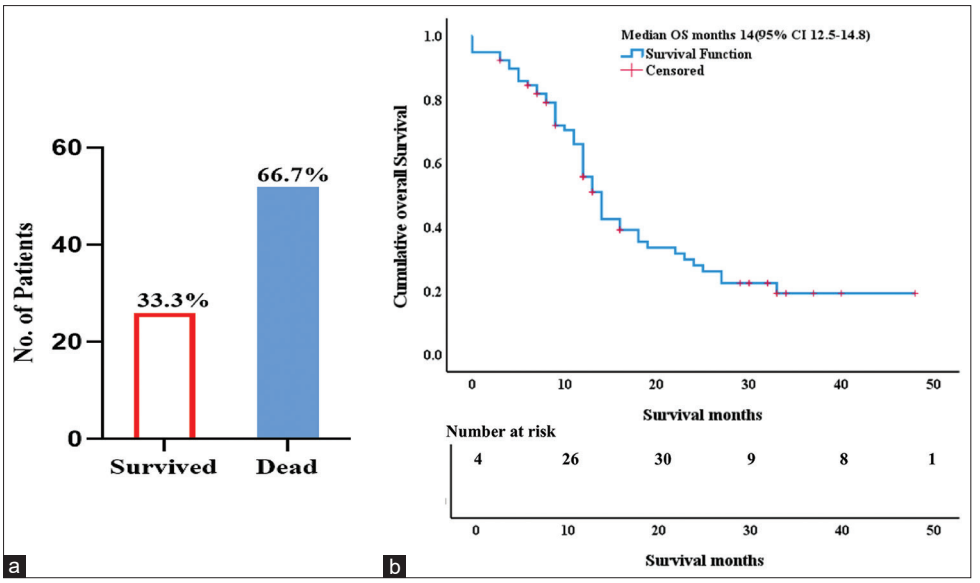

Figure 2: (a) Frequency of survival status of gastric cancer patients. (b) Kaplan-Meier curve of median overall survival of the entire studied GC population

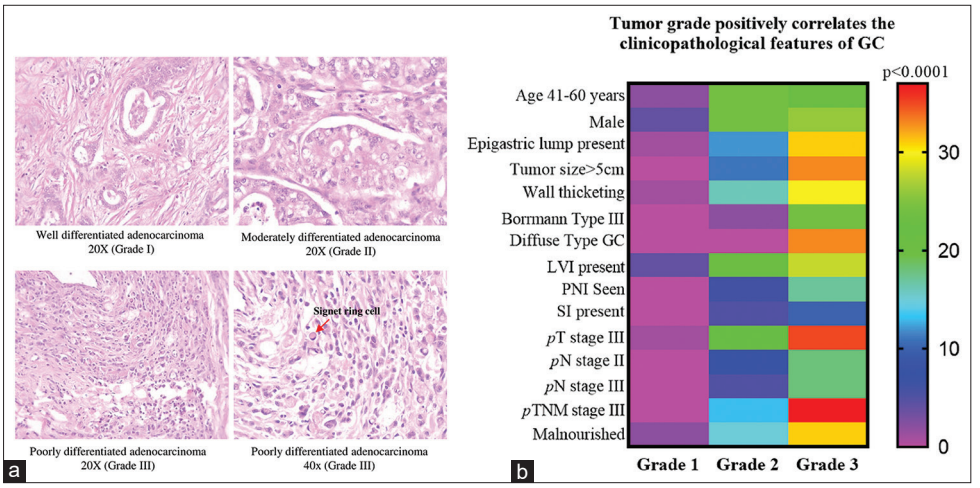

Figure 3: (a) The plates showing hematoxylin and eosin staining (20X; 40X) of tumor grade differentiation of GC patients. (b) Heat map representing the significant correlation between tumor grades and different clinicopathological features

subtotal, proximal, and majorly distal gastrectomy with 54 patients (69.2%). The adjuvant chemotherapy regimen consisted of capecitabine and platinum-based oxaliplatin 45 (91.8%) while the FLOT regimen was utilized for NACT. The overall response rate (ORR) was 43.6%, with 44 patients (56.4%) classified as non-responders (NRs). Complications post-treatment were observed in 52 patients (66.7%), predominantly due to poor nutritional status (48 patients, 61.5%). Recurrence was noted in 11 patients (10.3%), with local recurrence occurring more frequently than regional recurrence. The patients receiving NACT with FLOT regimen, experienced a lower mean OS of 17.7 months [95% CI: 11.9–23.08], while those receiving capecitabine and oxaliplatin as ACT had a higher mean OS of 21.7 months [95% CI: 17.1–26.5]. The log-rank test indicated significant differences in mean OS between the chemotherapy regimens ($P < 0.001$). Additionally, FLOT-treated patients showed an HR ratio of 23.5 [95% CI: 6.3–88.2], suggesting a significantly higher hazard risk of death among patients ($P < 0.001$). Moreover, patients who received 4 cycles of ACT had an increased mean OS of 24.6 months [95% CI: 16.1–33.1] than patients who received 12 cycles ($P < 0.001$). The increasing number of cycles correlated with an HR of 1.26 [95% CI: 0.28–5.67] ($P < 0.001$) [Table 3].

DISCUSSION

The current study observed varying effects of clinicopathological factors and treatment outcomes on GC survival. Older age, confounding symptoms, administration of neoadjuvant FLOT chemotherapy, and number of chemotherapy cycles significantly impacted the overall survival rate.

Table 2: Clinicopathological features with survival and hazard risk in gastric cancer patients

Characteristics (*N*=78)		Frequency *n* (%)	Kaplan–Meier survival (univariate)			Cox regression survival (Multivariate)		
			OS (months)	95% CI	*P*	HR	95% CI	*P*
Age (Years)	19–40	14 (17.9)	18.05	10.27–26.34	<0.001	2.003	1.2–2.3	0.002
	41–60	47 (60.3)	23.28	18.44–29.17				
	>60	20 (21.8)	10.67	6.78–14.62				
Gender	Male	54 (69.2)	17.84	13.49–22.18	0.135	1.57	0.849–2.90	0.147
	Female	24 (30.8)	20.18	15.49–24.87				
Diet	Mixed diet	69 (88.5)	20.39	16.15–24.62	0.557	0.803	0.377–1.71	0.569
	Vegetarian	9 (11.5)	15.55	7.47–23.63				
Primary tumor site	Antrum	47 (60.3)	17.71	13.76–21.76	0.054	0.977	0.83–1.13	0.769
	Pylorus	10 (12.8)	13.02	6.78–19.27				
	Body	13 (16.6)	22.12	13.79–30.45				
	Lesser curvature	2 (2.6)	15.0	9.12–20.88				
	Fundus	2 (2.6)	4.50	3.52–5.82				
	Gastroesophageal junction	4 (5.1)	22.75	7.01–38.48				
Tumor size at diagnosis	≤5 cm	34 (43.6)	15.62	11.8–19.4	0.297	1.30	0.43–1.3	0.344
	≥5 cm	44 (56.4)	22.5	16.5–28.4				
Lauren classification	Intestinal type	45 (57.7)	19.20	14.12–24.31	0.392	0.791	0.53–1.6	0.406
	Diffuse type	33 (42.3)	18.07	13.45–23.95				
WHO classification	Tubular adenocarcinoma	37 (47.4)	20.02	14.54–25.50	0.458	0.883	0.72–1.22	0.351
	Mucinous adenocarcinoma	6 (7.7)	9.50	4.61–14.38				
	Signet-ring cell carcinoma	32 (41.0)	16.61	12.14–21.09				
	Poorly cohesive carcinoma	3 (3.8)	29.00	13.75–44.24				
Grade	I	4 (5.1)	19.5	2.2–36.7	0.650	1.15	0.72–1.8	0.557
	II	29 (37.2)	20.0	13.9–26.2				
	III	45 (57.7)	17.2	12.9–21.5				
Lymphovascular invasion (LVI)	Present	59 (75.6)	19.01	14.18–23.84	0.296	0.723	0.38–1.3	0.288
	Absent	19 (24.4)	20.26	15.14–25.37				
Perineural invasion (PNI)	Seen	23 (29.5)	17.5	13.0–22.0	0.585	1.17	0.64–2.1	0.545
	Not seen	55 (70.5)	19.2	14.4–24.0				
Serosal invasion (SI)	Present	15 (19.2)	24.6	14.9–34.3	0.319	1.42	0.69–2.9	0.520
	Absent	63 (80.8)	16.4	13.2–19.6				
pT-stage	T1(a, b)	2 (2.6)	32.6	25.7–39.6	0.355	1.32	0.82–1.8	0.194
	T2	8 (10.3)	16.7	7.3–26.1				
	T3	57 (73.3)	17.7	13.2–22.3				
	T4(a)	11 (14.1)	19.7	10.9–28.4				
pN-stage	N0	21 (26.9)	23.1	15.9–30.3	0.287	1.24	0.97–1.6	0.082
	N1	8 (10.3)	18.8	10.2–27.4				
	N2	26 (33.3)	17.4	11.9–22.9				
	N3 (a, b)	23 (29.5)	12.6	7.9-17.3				
pTNM stage	I	6 (7.7)	29.0	22.0–35.9	0.159	1.46	0.93–2.2	0.081
	II	22 (28.2)	18.1	11.6–24.7				
	III	50 (64.1)	16.5	12.3–20.7				

OS-Overall survival; HR-Hazard risk; CI-Confidence interval; *P*<0.05 is considered significant

Clinicopathological features like weight loss, large tumor size, higher tumor grade (Grade III), perineural invasion, serosal invasion, and advanced stage (stage III) were associated with a notable hazard risk of death. Additionally, compromised treatment outcomes further elevate the hazard risk leading to shorter overall survival.

The demographic analysis of the cohort revealed a median age of 52 ± 11.35 years, with a predominance of males (69.2%) from rural areas (74.8%). The incidence was primarily apparent in the middle-aged group, with 60.3% of patients falling between the ages of 41 and 60 years. The incidence of patients was majorly observed in the middle-aged group, ranging between 41 and 60 years (60.3%). However, the mean age (between 56 and 59 years), gender distribution (2:1 males to females) and patient habitation align with the findings of the studies conducted in the North-East (Mizoram, Manipur), as well as in North and South India.[15,16] In contrast, various countries in the Western world, Europe, and Eastern Asia reported comparable outcomes, highlighting an increasing incidence, and predominance of males, particularly in Japan, Mongolia, and the Republic of Korea, for both <60 and over the age of 60 years.[17]

The mode of dietary patterns, addiction habits, and personal family history significantly influence the risk of GC.[18-20] In this study cohort, patients largely consumed a mixed diet, consisting of non-vegetarian options with high salt intake, and many were addicted to smoking, tobacco, and alcohol. Some individuals had a family history of chronic gastritis or malignancy, as well as a personal history of peptic disorders. These factors emphasize the connotation between lifestyle choice, predisposition, and genetic susceptibility, explicated by the oncogenic diet and smoking. Studies from North-Eastern India and Kashmir have shown a relative risk of GC associated

with the intake of salted, fermented, and smoked meats, tobacco use, alcohol consumption, poor drinking habits, and a family history of cancers, which aligns with the findings from Western countries.[8,21-23]

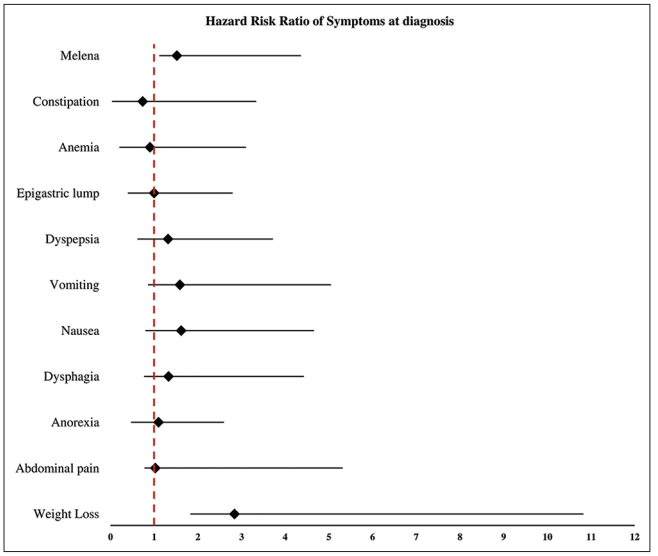

Figure 4: Initial symptoms of GC patients at diagnosis with overall survival and hazard risk

Our study observed an increasing proportion of diffuse-type GC incidence, although the frequency of intestinal-type GC remained comparatively higher among patients, consistent with recent literature worldwide.[24-27] Additionally, the antrum being the most prevalent site of tumor growth in our cohort, also aligns with previous evidence.[28-30]

The mean overall survival (OS) of the study population was 19.7 months in this cohort. Another study from North India reported a mean survival of 20.01 months in GC patients.[31] The age-stratified analysis in our study discerns a significant difference in survival among the three age groups, with older individuals experiencing increasingly shorter OS and a two-fold higher mortality risk than the younger individuals. In our study, gender, mixed diet, and addiction habits were recognized as non-significant risk factors, which contradicts the reports from other literature.[7,32,33] The consensus on the age-standardized incidence and mortality of GC has shown a declining range from 2.5% to 56.8% in the younger population of the UK, Sweden, the Equator, and Thailand,[34] whereas other reports, indicated that the non-metastatic patient have demonstrated an improved 5-year survival rates.[35] We identified the antrum as the most prevalent site of tumor growth. Interestingly, tumors sized ≥5 cm counterintuitively

Table 3: Treatment outcomes on survival and hazard risks in gastric cancer patients

Characteristics (*N*=78)			Frequency *n* (%)	Kaplan–Meier survival (univariate)			Cox regression survival (Multivariate)		
				OS (months)	95% CI	*P*	HR	95% CI	*P*
Treatment modality	Upfront surgery followed by adjuvant chemotherapy (ACT)		49 (62.8)	20.1	15.2–25.1	0.582	1.16	0.64–2.00	0.593
	Neo-adjuvant chemotherapy (NACT)		29 (37.2)	17.7	11.9–23.04				
Mode of gastrectomy	Total gastrectomy		5 (6.4)	16.8	6.2–27.3	0.282	1.00	0.76–1.33	0.961
	Subtotal gastrectomy		14 (17.9)	17.3	9.8–24.7				
	Proximal gastrectomy		5 (6.4)	11.0	3.8–18.1				
	Distal gastrectomy		54 (69.2)	19.9	15.2–24.7				
Chemotherapy regimen	ACT	Oxaliplatin and Capecitabine	45 (91.8)	21.7	17.1–26.5	<0.001	1.36	0.76–2.44	<0.001
	NACT	FLOT (5-FU\leucovorin, oxaliplatin and docetaxel)	29 (37.2)	17.6	12.2–22.9		23.5	6.3–88.21	
	None		4 (5.1)	0.00	0.00–0.00		0.00	0.00–0.00	
Chemotherapy cycles	ACT	Nil	4 (8.2)	0.00	0.00–0.00	<0.001	1.26	0.28–5.67	<0.001
		2	9 (18.4)	20.9	11.1–30.8				
		3	5 (10.2)	7.2	4.7–9.7				
		4	5 (10.2)	24.6	16.1–33.1				
		6	19 (38.8)	22.5	16.4–28.6				
		8	3 (6.1)	16.2	6.7–25.2				
		12	4 (8.2)	16.00	9.1–23.3				
Overall treatment response	Complete Responders (CRs)		17 (21.8)	19.50	13.7–24.0	0.289	1.22	0.87–1.7	0.235
	Partial Responders (PRs)		17 (21.8)	24.57	13.3–34.1				
	Non-Responders (NRs)		44 (56.4)	16.09	11.6–20.2				
Treatment response of ACT patients	Complete Responders (CRs)		9 (20.0)	22.3	16.5–28.1	0.598	1.54	0.622–3.8	0.618
	Partial Responders (PRs)		13 (28.8)	23.7	12.4–35.01				
	Non-Responders (NRs)		23 (51.1)	17.4	12.1–22.6				
Treatment response of NACT patients	Complete Responders (CRs)		8 (27.5)	14.7	7.1–22.3	0.726	0.56	0.05–3.5	0.743
	Partial Responders (PRs)		4 (13.7)	20.0	3.9–36.0				
	Non-Responders (NRs)		17 (58.6)	16.5	9.6–23.4				
Nutritional status after chemotherapy	Good		8 (10.3)	22.02	15.9–29.6	0.373	1.32	0.81–1.83	0.174
	Average		22 (28.2)	22.17	14.5–29.8				
	undernourished		48 (61.5)	16.49	12.7–20.6				
Post treatment complications	Present		52 (66.6)	18.71	13.8–23.7	0.328	0.970	0.54–1.72	0.917
	Absent		26 (33.3)	19.44	14.8–24.2				

N=Number of individuals; %=percentage; *P* value is significant at <0.05

exhibited a higher mean survival time compared to those sized <5 cm, which contradicts established associations between smaller tumor size and longer survival, as well as findings that identify smaller tumors as an independent risk factor for tumor deposits.[36] This discordance could be due to other confounding features present in patients with smaller tumors. There were no significant differences in survival between patients with intestinal and diffuse gastric cancer. As a tertiary care centre, this hospital-based study primarily diagnosed the patients with grades III, and IV, notably having signet ring cell carcinoma. The grade-III tumors positively correlated with older age, males, large tumor size, stomach wall thickening, Borrmann ulcerative carcinoma, diffuse type, LVI, pT stage, pN stage, and undernourishment. Deaths were more prominent among grade III patients; however, they exhibited a non-significant trend towards worse survival. In the literature, an association was observed between poorly differentiated grades and pathological variables like LVI, nodal status, and pTNM stage, reaffirming their negative prognostic influence. Signet ring cell carcinomas typically present poorly differentiated tumor cells, which are associated with worse prognosis and increased risks of metastasis, adversely affecting overall survival.[37-39]

In the present study, the choice of chemotherapy regimen emerged as a significant factor influencing the overall survival of GC patients. Those receiving preoperative FLOT, appeared with significantly elevated hazard risk, resulting in compromised survival months. Interestingly, the overall survival of the neoadjuvant FLOT recipients decreased with an increasing chemotherapy cycle, whereas survival improved with increasing cycles of adjuvant CAPOX chemotherapy. Research conducted in Eastern India also reported improved survival in patients undergoing surgery compared to other treatment modalities.[31] On the contrary, perioperative FLOT and its modified versions have shown better response rates and survival compared to other chemotherapeutic regimens in patients from different regions of India and around the world.[13,40-42] The chemotherapy responses among patients were heterogeneous, irrespective of the chemotherapy regimen. Most patients were non-responders, experiencing shorter survival months in either chemotherapy group. The risk of death, as indicated by different response categories (CRs, PRs, and NRs), showed no significant distinction between them. Nonetheless, in other findings, the treatment response and OS of FLOT-treated patients with signet ring cell carcinoma were not significantly different than those who underwent surgery.[43,44] The disparities observed in our cohort may be attributed to the positive correlation between treatment modalities and clinical factors such as older age, advanced tumor stage, epigastric lump (54.4%), anemia (61.5%), weight loss (88.5%), type of gastrectomy (mainly total, proximal, and subtotal; 31%), and post-operative complications (66.6%) like undernourishment. Patients who followed a mixed diet, reported severe weight loss and poor performance status at diagnosis, subsequently leading to undernourishment after chemotherapy. Specifically, the majority of the neoadjuvant FLOT-receiving individuals were undernourished compared to those adjuvant CAPOX-receiving patients. As observed, neoadjuvant FLOT chemotherapy resulted in significantly lower survival, partly due to higher toxicity and postoperative complications. These interrelated factors adversely accelerated the mortality, contributing to compromised survival rates in GC.

In our country, particularly in the state of West Bengal, where there are significant resource constraints in terms of access to tertiary healthcare for early diagnosis, adequate surgery, and chemotherapy, along with a shortage of qualified medical oncologists to manage chemotherapy-related toxicities (such as the highly toxic FLOT regimen), these treatments may not be as suitable as in other parts of the world. Future studies should focus on developing newer, less toxic yet equally effective neoadjuvant chemotherapy protocols.

The study offers important insights of the demographic and clinicopathological characteristics of GC patients in eastern India and indicators of risk stratification in an underrepresented population. Despite the study's identification of important associations, such as the link between poor survival and age, lymph node invasion, NACT with FLOT, and increased adjuvant CAPOX chemotherapy, limitations like small sample size and challenges in distinguishing treatment responses hinder the generalizability of the findings. Additionally, the influence of malnourishment on non-responder patients warrants further exploration. Furthermore, a substantial number of defaulted patients and a short follow-up period limited and hindered the observations during the study introducing bias and accuracy of the results.

CONCLUSIONS

In this study, poor survival and increased hazard risk were significantly associated with older age, extensive lymph node invasion, neoadjuvant FLOT chemotherapy, and chemotherapy cycles. While certain clinicopathological features like tumor size, grade, stage, and progressive weight loss, demonstrated no significant impact on survival. Notably, NACT with FLOT exhibited compromised survival and significant hazard risk in non-responder undernourished patients. Conversely, a greater number of patients who had undergone successful D2 gastrectomy and adjuvant CAPOX chemotherapy cycles were associated with improved survival in GC patients of eastern India.

Financial support and sponsorship

This work was funded by Chittaranjan National Cancer Institute, Kolkata, West Bengal, India.

Conflicts of interest

There are no conflicts of interest.

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Medical Oncology (2025) 42:29
https://doi.org/10.1007/s12032-024-02580-6

REVIEW ARTICLE

Sorcin: mechanisms of action in cancer hallmarks, drug resistance and opportunities in therapeutics

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Received: 12 October 2024 / Accepted: 3 December 2024

Abstract

Soluble resistant related calcium binding protein (Sorcin) plays an important role in tumor progression, angiogenesis, metastasis, and multidrug resistance. Differential expression of Sorcin across different cancers significantly correlates with key clinicopathological characteristics and survival outcomes, underscoring its potential as a prognostic marker. Its involvement in drug-resistant cancers further advert Sorcin as a promising therapeutic target. This review summarizes the mechanistic role of Sorcin in cancer, its contribution to drug resistance, clinical relevance, and the current and emerging therapeutic approaches aimed at translating Sorcin-targeted therapies into clinical practice.

Keywords Sorcin · Cancer · Multidrug resistance · Signaling pathways · Therapeutics · Clinicopathology

Abbreviations

ABC	ATP binding cassette
Akt	Ak strain transforming (protein kinase B)
ANXA	Annexin A1
ALDH1A1	Aldehyde dehydrogenase 1A1
Bcl2	B-cell lymphoma 2
Bax	Bcl-2–associated X protein
BCR-ABL	Breakpoint cluster region-Abelson proto-oncogene
CDK	Cyclin dependent kinase
CREB	Cyclic AMP-Responsive Element-Binding Protein
CTSZ	Cathepsin Z
Caspase	Cysteine-aspartic proteases
c-myc	Cellular myelocytomatosis
c-fos	Cellular Finkel–Biskis–Jinkins murine osteogenic sarcoma virus
c-jun	Cellular Ju-nana (the Japanese word for 17)
CHOP	Cyclophosphamide, doxorubicin, vincristine, prednisone
CRISPER cas9	Clustered regularly interspaced short palindromic repeats
ER	Endoplasmic reticulum
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinase
EPCAM	Epithelial cell adhesion molecule
EMT	Epithelial-mesenchymal transition
ECM	Extracellular matrix
EIF2	Eukaryotic Initiation Factor 2
FOX	Forkhead box
FLi1	Flightless-1
GATA	GATA Binding Protein
GSH	Gamma-glutamylcysteinylglycine
HIF	Hypoxia-inducible factors
IKK	Inhibitor of nuclear factor kappa B kinase
IAP	Inhibitor of apoptosis proteins
JAK	Janus kinase
KIT	Receptor tyrosine kinase
MAPK	Mitogen-activated protein kinase
MEK	Mitogen-activated protein kinase kinase
MMP	Matrix metalloproteinase
mTOR	The mammalian target of rapamycin
MDR	Multidrug resistance
MRP	Multidrug resistance protein
NF-κβ	Nuclear factor kappa-light-chain-enhancer of activated B

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Published online: 14 December 2024

NLRP3	NLR family pyrin domain containing 3
NFAT	Nuclear factor of activated T-cells
PI3K	Phosphatidylinositol-3 kinase
PDCD	Programmed cell death
PARP	Poly-ADP ribose polymerase
PTX	Platinum resistance
PROTAC	Proteolysis-targeting chimeras
RB	Retinoblastoma
ROS	Reactive oxygen species
STAT3	Signal transducer and activator of transcription 3
SOX9	Sex-determining region Y-Box Transcription Factor 9
Src	Sarcoma
SMAD	Suppressor of Mothers against Decapentaplegic
SNIPER	Specific and nongenetic IAP-dependent protein erasers
TNFα	Tumor necrosis factor-α
TNBC	Triple negative breast cancer
TRAP 1	Tumor necrosis factor receptor-associated protein
TP53	Tumor protein p53
VEGF	Vascular endothelial growth factor
Wnt	Wingless-related integration site
ZEB1	Zinc finger E-box binding homeobox 1

Introduction

Over the years, research has identified numerous intriguing cancer-associated targets or biomarkers, which regulate the onset and progression of cancer, offering significant diagnostic and therapeutic value [1, 2]. Emerging therapies targeting these markers have shown promising therapeutic potential across various cancer types [3, 4]. However, certain cancers and subtypes- such as gastric, pancreatic, ovarian, TNBC, sarcoma, glioblastoma, and neuroendocrine tumors- still lack reliable or well-developed biomarkers [5, 6]. Notably, Soluble Resistance-Related Calcium Binding Protein (Sorcin) has recently gained attention for its unique structure and functional significance in cancer.

Sorcin is a 198-amino acid protein, encoded by the SRI gene, which contains five E–F hand domains, organized into eight exons and is activated upon calcium binding [7, 8]. Sorcin is primarily localized in cytoplasm and translocate to sarcoplasmic reticulum, mitochondria, nucleus and plasma membrane, particularly in response to calcium elevation [9]. Sorcin plays a critical role in cardiovascular, neurological diseases, and cancer, and is predominantly involved in calcium homeostasis [10]. In cancer, Sorcin is emerging as potential onco-protein, increasingly recognized for its role in cancer progression and chemoresistance. It is known to regulate key mechanisms including initiation, progression, metastasis, and survival [11]. Interestingly, the SRI gene shares the same chromosomal locus, 7q21.12, with several ABC transporter genes and co-amplify with MDR1 protein in multidrug-resistant (MDR) cancers [12]. Evidently, Sorcin is often induced in MDR cells by therapeutic agents like vincristine, doxorubicin, 5-fluouracil among others [13]. More precisely, it is the MDR property of Sorcin that is gaining significant consideration, given its high binding propensity for both oncogenic proteins and anticancer drugs, making it an important target in cancer therapeutics [14, 15].

This updated review comprehensively focuses on the mechanistic role of Sorcin across various cancer hallmarks, its contribution to multidrug resistance, and its clinical significance. It also explores current, and emerging therapeutic strategies targeting Sorcin, highlighting the rationale for advancing Sorcin-targeted therapies into clinical trials while identifying existing gaps in research.

Involvement of sorcin in cancer

Sorcin is differentially expressed across various cancers, acting as an oncoprotein in some while its role remains uncertain in others. Sorcin is overexpressed in several sporadic cancers, including glioblastoma multiforme, nasopharyngeal, breast, lung, gastric, gallbladder, colorectal, hepatocellular, bladder, ovarian, as well as leukemia and myeloma, as demonstrated in various tumor models [16–24]. Furthermore, Sorcin expression cornerstones mechanisms that encompasses aggressive clinical features and distinct stages of tumor development including initiation, progression, epithelial mesenchymal transition (EMT), migration, invasion, angiogenesis, metastasis and metabolism while inhibiting programmed cell death [21, 25–28].

Mechanism of sorcin in cell cycle

The progression of the cell cycle is a tightly regulated process, orchestrated by important oncogenes and tumor suppressor proteins [29, 30]. There are evidences of Sorcin interacting proteins involved in cell cycle, particularly, mitosis and cytokinesis, including AURKA/B, PLK-1, SYL1, ANXA11, PSRC1, CACNB1, among others [31, 32]. Although a few studies have highlighted the influence of Sorcin on the cell cycle, the understanding of how Sorcin acts with these cell cycle regulators remains limited [33, 34].

Mechanistically, Sorcin suppression at both transcriptional and translational levels has been shown to reduce the expression of p21, cyclin D1, c-Myc, and phospho-Src, while activating p53. These alterations led to changes in the distribution of cells across different cell cycle phases. Specifically, Sorcin silencing results in cell cycle arrest in

both the G0/G1 and G2/M phases [35, 36]. However, the exact mechanisms by which Sorcin exerts its influence at each phase of the cell cycle remained dubious.

Furthermore, the role of Sorcin in cell division come from its cellular localization. In NRK, 3T3-L1 fibroblasts, 293FT, Cos7, and Huh7 cells, Sorcin localizes and interacts targets, and participates in the phosphorylation-dephosphorylation network important for mitosis and cytokinesis. Sorcin localized in the nucleus during interphase. However, during prophase and metaphase, Sorcin-containing vesicles accumulates in the apical region of the mitotic spindle, and in anaphase, it is concentrated in the central zone of the spindle. By late telophase, these vesicles are predominantly found in the midbody. Notably, Sorcin colocalizes with key regulators such as Aurora A, Aurora B, and Polo-like kinase 1 (PLK1) in the cleavage furrow and midbody, facilitating successful cytokinesis (Fig. 1). Mechanistically, Sorcin is believed to harbor active sites for Aurora A, Aurora B, and PLK1, with phosphorylation playing a critical role in its function. Thr155, in particular, is a key phosphorylation site targeted by PLK1. Mutation of Thr155 to aspartic residues reduces this phosphorylation. Ser149 and Thr150 are also crucial for PLK1-mediated phosphorylation of Sorcin. Upon activation, Sorcin interacts directly with PLK1 in a calcium-dependent manner, regulating PLK1's kinase activity through autophosphorylation. This suggests a pivotal role for Sorcin in coordinating cell cycle progression through its regulation of PLK1 and other mitotic.

kinases [10].

The results suggest the cell cycle regulation by Sorcin through calcium signaling to vesicle trafficking and interactions with key regulators. While its role in mitotic and cytokinesis progression is known, its interaction with the cyclin-dependent kinases (CDKs), spindle assembly checkpoint (SAC) proteins—such as CDK4/6, MAD and BUB, crucial in cancer progression and drug resistance [37, 38]-remains unexplored. Therefore, understanding the core mechanism of Sorcin modulating these CDKs and SAC proteins could fill a crucial gap for cancer therapies aimed at overcoming drug resistance through cell cycle control.

Mechanism of sorcin in tumor progression

In cancer progression, Sorcin influences signaling molecules involved in cell proliferation, EMT, survival, and angiogenesis-associated signaling pathways involving MAPK/ERK, PI3K/Akt, JAK/STAT, ANXN, and VEGF pathways [19, 21] (Fig. 2). Specifically, Sorcin regulates EGF-dependent EGFR signaling pathway. Sorcin directly binds to EGFR in a calcium-dependent manner, enhancing EGFR proteostasis and phosphorylation. By stabilizing EGFR, Sorcin induces hyperphosphorylation and

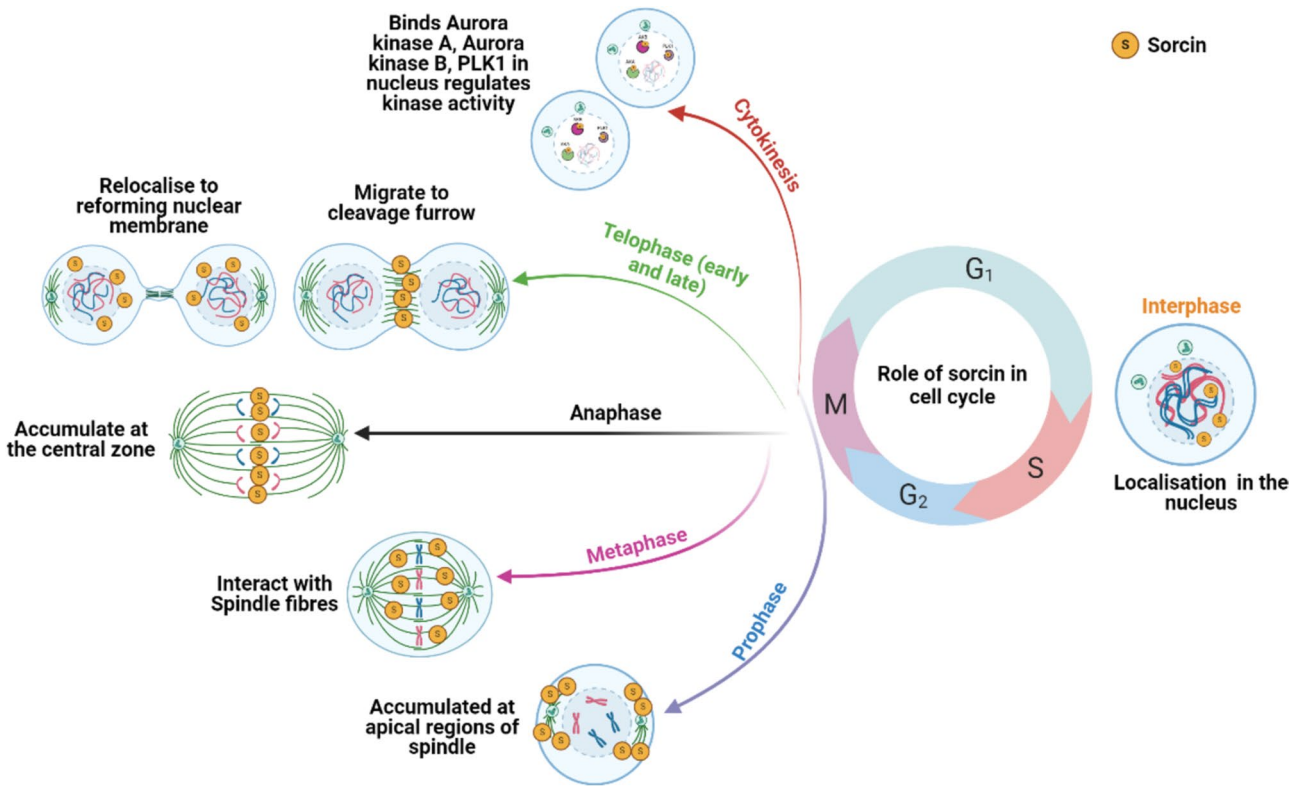

Fig. 1 Role of Sorcin in various phases of the cell cycle and its interaction with AURKA/B and PLK1 [10]

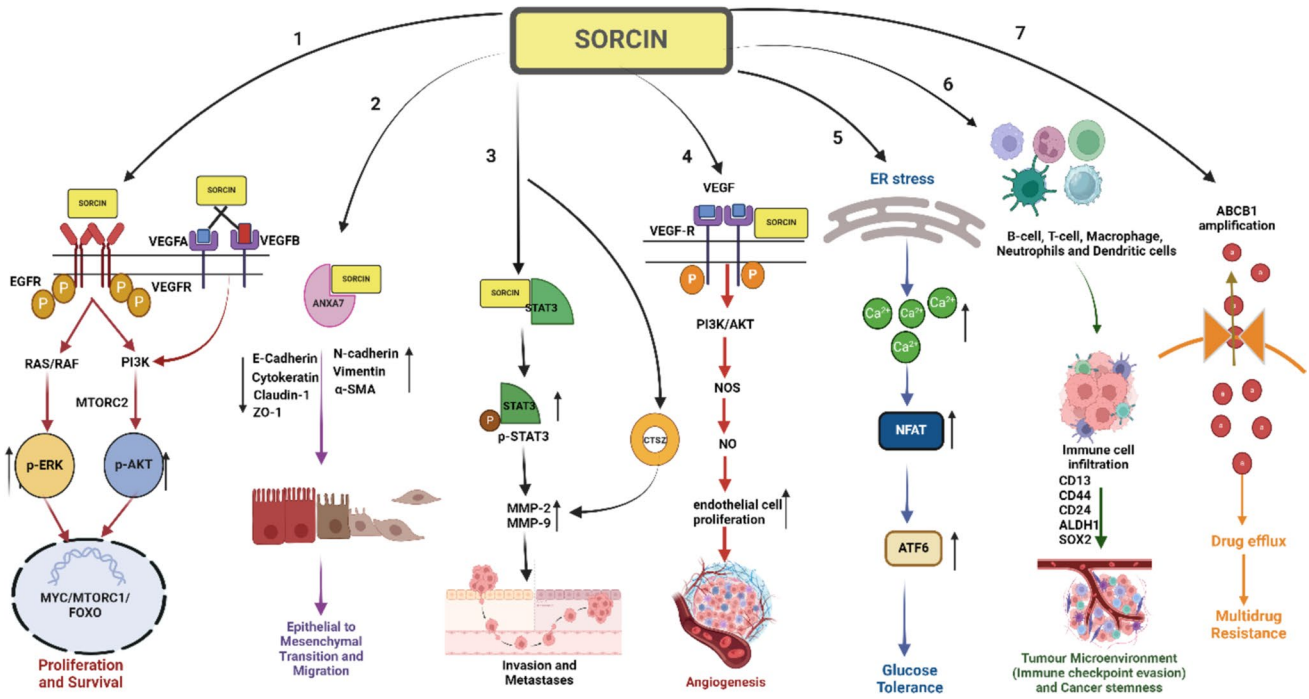

Fig. 2 Sorcin regulating signaling pathways in cancer. 1. Sorcin overexpression in pancreatic β-cells improves glucose tolerance by increasing cytosolic flux and ER Ca^{2+} storage, activating NFAT and ATF6 transcriptional activity. 2. Sorcin regulates cancer cell proliferation and survival through the Sorcin-EGFR/pERK/pAKT/mTOR and Sorcin/VEGFA/B-VEGFR/PI3K/AKT pathways. 3. Sorcin interacts with ANXN7, upregulating EMT and migration-related factors, while downregulating N-cadherin, vimentin, and α-SMA levels. 4. Sorcin physically binds to STAT3, activating MMP2/9 and CTSZ to drive invasion and metastasis. 5. Sorcin promotes angiogenesis by proliferating endothelial cells via the Sorcin/VEGF-VEGFR/PI3K/pAKT/NOS/NO pathway. 6. Sorcin further activates immune cells and cancer stem cells (CSCs) to modulate the tumor microenvironment and maintain cancer stemness. 7. In MDR cancers, Sorcin overexpression induces P-gP upregulation (ABCB1 gene) thereby enhancing drug efflux while promoting drug resistance. ↑ = Upregulation; ↓ = Downregulation

subsequent activation of downstream RAS/RAF/MEK/ERK and PI3K/AKT signaling cascades [39]. In HCT116 cells, Sorcin downregulates the epithelial marker E-cadherin and upregulates mesenchymal markers like vimentin, N-cadherin, fibronectin, and α-SMA, enabling cell motility and invasiveness. Moreover, Sorcin drives metastasis, in part, by activating the PI3K/Akt/mTOR signaling pathway, while promoting cell migration, invasion, and the EMT process [28]. Beyond the PI3K/Akt/mTOR pathway, Sorcin also extends its mechanism to JAK/STAT pathway. Sorcin binds to STAT3, triggering its activation further enhancing metastatic potential through overexpression. Sorcin promotes the expression of ANXA7, MMP2, and MMP9, key factors involved in extracellular matrix degradation and metastasis [21, 22, 40]. Additionally, it activates CTSZ signaling in gastric cancer, facilitating metastasis while degrading the extracellular matrix [21]. Furthermore, Sorcin is associated with microsatellite instability (MSI), tumor mutational burden (TMB), Claudin-1, ZO-1, and E-cadherin, which collectively contribute to EMT and increased metastatic capabilities [41]. Moreover, In MSI-H tumors, mutated or dysregulated Sorcin may promote aggressive behavior and immune evasion.

In early-stage angiogenesis in hepatocellular cancer, characterized by high vascular density, Sorcin regulates VEGFA/B and VEGFR1 levels. VEGFA/B binding to VEGFR1 promotes cell proliferation via the PI3K/Akt/FOXO1 axis [42]. Additionally, Sorcin engages in Ca^{2+}-mediated angiogenesis through the VEGF/PI3K/Akt and NO pathways, crucial for tumor vasculogenesis in endometrial cells. By inducing VEGF expression, Sorcin triggers the PI3K/Akt cascade, leading to NOS activation, which increases NO levels and facilitates endothelial cell proliferation, migration, and invasion, driving angiogenesis [27].

Mechanism of sorcin in glucose metabolism

Sorcin is chiefly essential for maintaining normal glucose tolerance, a significant risk factor in certain cancer types. Mechanistically, its calcium-regulating function directly influences glucose-stimulated insulin secretion (GSIS) by ensuring proper calcium signaling in pancreatic β-cells. Under lipotoxic conditions, its expression decreases, leading

to β-cell dysfunction and glucose intolerance. However, Sorcin overexpression stabilizes ER calcium levels, reduces ER stress markers (CHOP, GRP78/BiP), and protects β-cell function. Additionally, Sorcin downregulates G6PC2, a negative regulator of insulin secretion, enhancing GSIS under high-glucose conditions, further activating NFAT, and stimulating ATF6 transcription, influencing cellular metabolism and enhancing β-cell resilience [43–45].

Sorcin controlling cancer stemness and tumor microenvironment

Sorcin is associated with cancer stemness, and the immune landscape of the tumor microenvironment. Its the mRNA expression (SRI) correlates with cancer stemness markers, CD133, CD44, CD24, SOX2 and ALDH1, which are crucial for maintaining stem cell-like properties in tumors. Sorcin is positively related to tumor infiltration levels of B-cells, CD4 + T, CD8 + T cells, neutrophils, macrophages, and dendritic cells of tumor immune microenvironment, primarily across different cancer types [41].

Although significant progress has been made in understanding the role of Sorcin in cancer progression, the precise role of Sorcin need to be elucidated in various pathological development of cancer. Sorcin in both cancer progression and metabolism presents an interesting paradox. On one hand, it plays a protective role in glucose homeostasis against obesity, while on the other, it promotes cancer aggressiveness through various signaling pathways. This duality raises the question of how differently Sorcin regulates mechanisms between normal physiological processes and cancer. Addressing these gaps will enhance our understanding of Sorcin as a potential therapeutic target.

Mechanism of sorcin in programmed cell death

Sorcin employs distinct mechanisms to modulate different cell death pathways, imparting its protective effect against cell death (Fig. 3).

Mitochondrial apoptosis regulation

Sorcin activates the NF-κB pathway through two mechanisms: it either binds to STAT3 or stimulates CAM KII, and cAMP-dependent protein kinase (PKA), thereby initiating tumorigenesis by preventing apoptosis. This interaction leads to the phosphorylation of NF-κB subunits, p65 and IκB, enhancing the expression of anti-apoptotic proteins

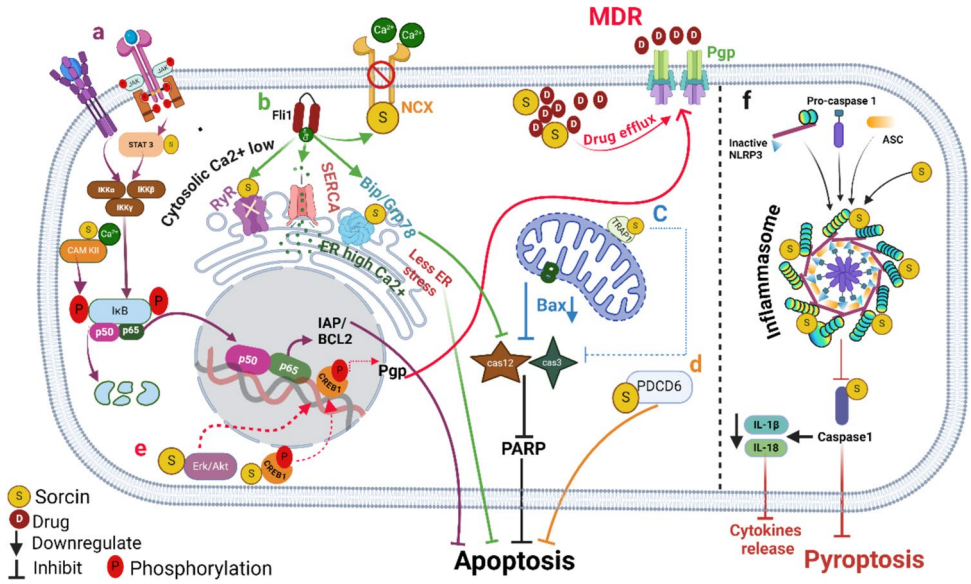

Fig. 3 Sorcin Regulating Programmed Cell Death events. Sorcin plays a crucial role in regulating programmed cell death through multiple mechanisms: Mitochondria-Mediated Apoptosis: **a** Sorcin binds to STAT3 and also activates CaMKII, to facilitate the NF-κB pathway. This results in the transcription of Bcl2 and IAP via the p50-p65 complex, inhibiting apoptosis. **b** Sorcin also maintains calcium homeostasis and alleviates ER stress in cancer cells, with the help of Fli1by inhibiting RyR and activating SERCA. Sorcin activates Bip/GRP78 thereby inhibiting Caspase 12. **c** Sorcin binds to TRAP1, inhibits Bax, Caspase 3, and PARP. **d** Sorcin interacts with PDCD6, impeding mitochondria-mediated apoptosis in both drug-sensitive and drug-resistant cancers. **e** Multidrug Resistance: Sorcin activates ERK/AKT signaling, which leads to the phosphorylation of CREB 1. This in turn promotes P-gP transcription, reducing intracellular drug concentrations, and thus inhibiting apoptosis in MDR cancers. **f** Pyroptosis Regulation: Sorcin binds to the inflammasome, inhibiting caspase-1 activity, thereby regulating pyroptosis

like Bcl-2, Bcl-XL, and MCL-1, while downregulating pro-apoptotic proteins like Bax and caspase-3 [46–48]. In MDR cancer cells, Sorcin also modulates the Bax and Bcl-2 levels, protecting the cells from the cytotoxic effects of chemotherapeutic agents like cisplatin. However, when Sorcin is downregulated, it leads to increased ROS production and mitochondrial membrane potential damage, ultimately triggering apoptosis through elevated level of cleaved caspase-3 and Bax [4, 49]. In a mechanism, 18-kDa isoform of Sorcin localize to mitochondria and binds the N-terminal end of TRAP1 protein, helping regulate mitochondrial permeability transition pore (MTP) opening, impedes the pro-apoptotic signaling molecules [50, 51]. Moreover, Sorcin linked to PDCD6, and PARP, in response to anticancer drugs to avoid apoptosis [49, 52, 53].

ER stress-induced apoptosis inhibition

Sorcin also interacts with the Sigma-1 receptor, in modulating ER-mitochondria communication and mitigating ER stress, facilitating calcium transfer between the organelles, which is essential for cellular bioenergetics and apoptosis regulation [54, 55]. Moreover, Sorcin interaction with Fli1 emphasizes a more nuanced regulation of ER stress-induced apoptosis. Fli1 modulates the activity of Sorcin by inhibiting RyR2 and RyR3, curbing excessive calcium release from the ER. Concurrently, Sorcin enhances calcium accumulation within the ER by activating SERCA, thereby reducing the risk of ER stress and preventing the activation of the unfolded protein response. This dual modulation of calcium dynamics—restricting its release while promoting its retention—supports cancer cells against stress-induced apoptosis [56].

Pyroptosis regulation

Recent studies have reported pyroptosis regulation by Sorcin, an inflammation-associated cell death. Sorcin interacts with NLRP3 inflammasomes, a protein complex involved in pyroptosis initiation. By modulating key components of the inflammasome, such as NLRP3, ASC, Caspase-1, P20-Caspase-1, IL-18, IL-1β, GSDMD, and GSDMD-N, thereby inhibiting the release of cytokines that would otherwise activate caspase-1-mediated pyroptosis. Through this inhibition, Sorcin prevents the inflammatory cascade and maintains cellular integrity [57]. Sorcin appears to regulate apoptosis and pyroptosis, while its impact on other cell death types remains obscure.

From regulating mechanisms such as mitochondrial permeability, ER-mitochondria communication, calcium dynamics, and pyroptosis, Sorcin serves as a multitargeted modulator. However, the emphasis on these specific pathways may open the potentially broader scope into its involvement across different PCD forms and more refined targeting strategies. Furthermore, developing Sorcin-targeted inhibitors that effectively disrupt its protective effects against apoptosis without affecting normal cells presents a significant challenge in the pursuit for effective cancer therapies.

Sorcin in intrinsic and acquired MDR

Sorcin binds therapeutic drugs, including gemcitabine, vinblastine, vincristine, doxorubicin, Daunorubicin, Adriamycin, Irinotecan, 5-fluouracil, platins, and taxanes, with high affinity, thereby enhancing resistance against these xenobiotics during cancer treatment [12, 58] (Table 1). Drug resistance can be either intrinsic, with pre-existing genetic mutations which exists before treatment or acquired, developing after chemotherapy while patients become unresponsive to the regimen [59, 60]. Initially, most recipients respond to chemotherapy because the majority of tumor cells are drug-sensitive; however, resistant subclones of the heterogenous tumor, often harboring insensitive cancer stem cells, can survive. These cells, coupled with the intrinsic defense pathways, proliferate aggressively post-treatment, leading to cancer relapse and early recurrence [61, 62]. Acquired resistance, on the other hand, develops through gradual activation of secondary proto-oncogenes like Sorcin, altered expression of drug targets, and changes in the tumor microenvironment following treatment. While the molecular mechanisms of intrinsic and acquired resistance differ, they may overlap or coexist during tumor progression and treatment [63]. The chromosomal location of Sorcin, its overexpression and co-amplification with P-gP, an important efflux transporter protein involved in MDR, indicates its role in intrinsic drug resistance [34, 64]. While P-gP knockdown cannot entirely reverse drug resistance, Sorcin overexpression, independent of P-gP, contributes significantly to chemoresistance and partially reverses the resistant phenotype [65]. Sorcin elevation could results from genetic mutations, epigenetic changes or conserved molecular mechanism in resistant cancer cells. Furthermore, Sorcin regulate calcium homeostasis differently in resistant cells, contributing to the broader context of drug resistance further enhancing overall drug resistance [4, 66, 67]. Acquired resistance is often an extension of intrinsic resistance, leading to tumor relapse and progression. In the past study, amplification of Sorcin did not possess collateral sensitivity or any cross resistance to chemotherapeutic drugs [68], a failed hypothesis as recently Sorcin upregulation against mono-therapeutic agent deliberates cross resistance to other administered chemotherapeutic drugs in various cancer models have been documented [24]. The observation is in agreement with the outcomes of Yamagishi et al. where the deliberate Sorcin elevation induces the co-amplification

Table 1 Overexpression of Sorcin in drug-resistant cancer cells in response to chemotherapeutic drugs

Sl. No	Chemotherapeutic drugs	Cancer	Tumor model	References
1	Gemcitabine	Non-small cell lung cancer (NSCLC); Gemcitabine NSCLC	H460 cells; H460/ GEM	[58]
2	Vincristine	Human Gastric Cancer; Vincristine resistant gastric cancer	SGC790; SGC790/VCR	[66, 69, 98–102]
		Leukemia cells; Adriamycin resistant leukemia cells	K562; K562/A02	
		Vincristine resistant leukemia cells	ARH77 Vin^R	
			U937 Vin^R	
		Ehrlich ascites cancer cells	EHR/VCR +	
		Hamster lung cancer cells; vincristine resistant hamster lung cancer cells	DC-3F; DC-3F/VCRd-5L	
		Colchicine resistant Chinese hamster ovary cells	CH^RC5	
		Chinese hamster ovary cells	AuxB1 cell line fractionated X-irradiation generated sublines designated DXR-10	
		Vincristine-resistant lymphoma cells	HOB1; HOB1/VCR1.0	
3	Daunorubicin	Ehrlich ascites cancer cells	EHR2/DNR +	[67, 103]
		Adriamycin resistant leukemia cells	K562/A02	
4	Actinomycin D	NA	QUA/ADj	[104]
		The Chinese Hamster Ovary cell line	hamster CHO subline	
5	Methotrexate	Human methotrexate leukemia cell line	HL60 MTX^R	[24, 98]
			U973 MTX^R	
		Methotrexate T lymphocyte cell line	Jurkat MTX^R	
		Leukemia	CCRF-CEM/MTX	
6	Adriamycin	Gastric Cancer; Vincristine resistant gastric cancer	SGC790; SGC790/VCR	[102–104]
		Leukemia cells; Adriamycin resistant leukemia cells	K562; K562/A02	
		Neuroblast cell	BE (2)-C/ADR	
7	Doxorubicin	Lung carcinoma cells	H1299; Calu-1; A459	[13]
		Adriamycin resistant leukemia cells	K562/AD2	
		Breast cancer cell	MDA-MB-468; MDA-MB-231	
		Adriamycin breast cancer cell	MCF7/AD2	
		Chinese hamster ovary cells	AuxB1	
		Doxorubicin resistant leukemia cells	ARHD60 DXR	
8	Cisplatin	Leukemia cells; Adriamycin resistant leukemia cells	A549/DDP	[13, 105, 106]
		Cisplatin resistant leukemia cell line	HL60 $cisPt^R$	
		Cisplatin resistant T lymphocyte cell line	Jurkat $cisPt^R$	
		Cisplatin resistant non-Hodgkin's B-cell line	DoHH-2 $cisPt^R$	
		Leukemia	Murine P388 subline	
		Djungarian hamster cell line	DM15 subline	
9	Oxaliplatin	Human colorectal cancer	Human HT-29 and HCT-116	[50]
10	Paclitaxel	Ovarian cancer cells; platinum resistant ovarian cancer cells	OVCAR 3; OVCAR3/PTX	[83, 85]
		Human Gastric Cancer; Vincristine resistant gastric cancer	SGC790; SGC790/VCR	

Table 1 (continued)

Sl. No	Chemotherapeutic drugs	Cancer	Tumor model	References
11	5-Fluorouracil (5-FU)	Human Gastric Cancer; Vincristine resistant gastric cancer	SGC790; SGC790/VCR	[50]
		Human colorectal cancer	Human HT-29 and HCT-116	
12	Irinotecan	Human colorectal cancer	Human HT-29 and HCT-116	[50]
13	Etoposide	Leukemia cells; Adriamycin resistant leukemia cells	K562; K562/A02	[107–109]
		Chinese hamster ovary cells	AuxB1 cell line fractionated X-irradiation generated sublines designated DXR-10	
14	Homoharringtonine	Leukemia cells; Adriamycin resistant leukemia cells	K562; K562/A02	[12]
15	CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone)	Diffuse large B-cell lymphoma (DLBCL)	Patients (Human model); CHOP resistant DLBCL cells	[110]
16	FLOT (5-FU/leucovorin; Oxaliplatin; Docetaxel)	Advanced Gastric cancer	Patients (Human model)	(Unpublished data of our laboratory)

of P-gP in response to anticancer drugs addressing the development of secondary (acquired) resistance [69].

The concrete difference between intrinsic and acquired resistance in context of Sorcin is still unclear in the literature. However. tumor cells exhibit elevated Sorcin expression might be an adaptive mechanism to evade apoptosis and ER stress, thereby supporting the development of MDR.

Mechanism of sorcin in the development of multidrug resistance (MDR)

Sorcin contributes to multidrug resistance in various cancers, most notably leukemia, breast, lung, gastric, ovarian, and glioblastoma, complicating treatment outcomes (Table 1).

Its behavior varies across different MDR cancers, exhibiting distinct responses to various drugs. In the onset of MDR, Sorcin was found to localize predominantly in the cytosol in response to doxorubicin while reducing its accumulation in other organelles. Mechanistically, it binds doxorubicin with high affinity in the cytosol, preventing its entry into the nucleus, and facilitates its efflux through the MDR1 pump (Fig. 2 **(7)**). This reduces intracellular accumulation of doxorubicin and inhibits its ability to mediate genotoxic topoisomerase II- DNA damage, free-radical generation, disruption of mitochondrial membrane potential, and PARP/Caspase pathway. Additionally, Sorcin's actions prevent mitotic catastrophe and cellular senescence [12]. In response to etoposide, overexpressed Sorcin modulates the expressions of P-gP, Bcl2 and Bax, hence protecting the cell from drug-induced apoptosis [70]. Sorcin also escalates the promotor activity of the mdr1 gene, by binding between 716 and 709 bp of CRE site, upstream of the mdr1 promoter. Meanwhile, it promotes the phosphorylation of the cAMP response element-binding protein1(CREB1) via PKA activation enhancing P-gP expression favoring drug efflux [69]. Moreover, Sorcin controls P-gP expression through ERK/Akt signaling pathways underscoring its unidirectional effect on P-gP regulation in resistant cancer cells [12, 49]. (Fig. 3e**).** Experimental downregulation of Sorcin, elevates E-cadherin while depleting ZEB-1, VEGF levels to inhibit, migration, invasion and angiogenesis thereby reversing Adriamycin resistance [71].

Despite the growing evidence of Sorcin as a significant contributor to MDR through its modulation of key signaling pathways, drug efflux mechanisms, and regulation of apoptotic factors, several gaps remain underexplored. Future research must address the inconsistent understanding of how Sorcin contributes to resistance mechanisms in cancer types with minimal P-gP activity. Additionally, there is also a lack of comprehensive studies exploring potential cross-talk between Sorcin and other MDR regulators. The literature does not fully address the role of Sorcin in CSCs, which are often linked to both intrinsic and acquired resistance and tumor relapse. However, the extent to which Sorcin specifically interacts with CSC markers and whether its inhibition could selectively target these cells remains uncertain. Moreover, distinguishing its precise role in intrinsic versus extrinsic MDRs could provide new therapeutic avenues to mitigate chemotherapy-induced cytotoxicity in cancer patients.

Role of sorcin in other diseases

Sorcin is highly expressed in the heart, particularly in cardiomyocytes and skeletal muscle tissues, where it plays a vital role in regulating calcium homeostasis and contractile

function. It interacts with the SERCA2a isoform in cardiac muscle cells. In cardiovascular conditions, Sorcin dysfunction can disrupt calcium handling, contributing to the development of hypertrophic cardiomyopathy and hypertension [72, 73]. Additionally, Sorcin physically interacts with the RyR in ER [15]. Studies have demonstrated that Sorcin inhibits RyR2 activity, thereby reducing calcium release from the ER. This inhibitory effect of Sorcin on RyR2 likely mediated by its direct binding to RyR2, stabilizing it in a closed state, and preventing calcium release [74]. The inhibition of excessive calcium release from the ER, prevents calcium overload and subsequent cellular dysfunction or apoptosis. The interplay between regulatory Sorcin, SERCAs, IP3Rs, and RyR provides a coordinated mechanism through which Sorcin regulates the amplitude and kinetics of calcium release events ensuring intracellular calcium homeostasis [75].

Sorcin also appears to be implicated in the pathogenesis, and progression of neurodegenerative diseases like Alzheimer's, Parkinson's, frontotemporal dementia, and Huntington's disease [74, 76–82]. Dysregulation of Sorcin has been found to alter calcium homeostasis in neurons and microglia cells, contributing to neuronal damage and cell death. In Alzheimer's disease, Sorcin counteracts the inhibitory effects of two major pathological components, amyloid-beta (Aβ) and tau, on plasma membrane calcium ATPase (PMCA) and effectively block Aβ- and tau-induced toxicity in human neuroblastoma cells while also preserving endogenous PMCA activity from their inhibitory effects [77]. Another feature of neurological disorders is oxidative stress, which is triggered by an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defense mechanisms. Sorcin acts as a ROS scavenger and regulates the activity of antioxidant enzymes, such as superoxide dismutase (SOD), thereby protecting neurons from oxidative damage [74].

Therapeutic importance of sorcin in cancer

As previously discussed, Sorcin is an appealing target in cancer treatment due to its role in drug resistance. Several strategies have been developed to target Sorcin, including natural phytochemicals, anticancer drugs, and RNA interference (RNAi) technologies, which have shown promise in recent years for overcoming Sorcin-mediated drug resistance (Fig. 4A).

ShRNA-mediated sorcin targeting

In vitro experiments have demonstrated that silencing Sorcin can modulate various regulatory proteins. For instance, it leads to the decrease in intracellular rhodamine 123 levels, cell cycle arrest at the G2/M phase, accumulation of rounded mitotic cells, and reduced cell growth and enhanced apoptosis. Furthermore, ShRNA-mediated Sorcin silencing downregulates MDR genes such as GST-π, Livin, Survivin, Src, Cyclin-D1, Bcl-2, C-myc, TP53, and p21,while also inhibiting the AKT/NF-κB signaling pathway by compromising Akt and NF-κB phosphorylation [35]. Furthermore, it reduces intracellular GSH levels in resistant cells, which plays a critical role in reversing drug resistance by downregulating the NF-κB/AKT pathway. Moreover, lipid-coated albumin-PTX nanoparticles loaded with Sorcin-specific siRNA (LANP-PTX-siSRI) have been shown to effectively inhibit Sorcin expression, restore intracellular calcium levels, promote apoptosis, and suppress the progress of PTX-resistant cancer cells [83].

mi-RNA mediated sorcin silencing

A study presented a therapeutic strategy to overcome drug resistance by targeting miR1/Sorcin signaling axis. Sorcin overexpression led to a significant downregulation of miR-1 level in cisplatin, Adriamycin- and vincristine-resistant cancer cells. Overexpression of miR-1 promote the degradation of Sorcin mRNA, partially reversing the drug resistance by inhibiting drug efflux pumps and the enhancing apoptosis through the suppression of Bcl-2 expression and the activation of c-fos, C-jun, and Bax [33, 84]. Furthermore, Sorcin silencing leads to the upregulation of PTEN expression, highlighting its potential as a therapeutic target in cancer treatment [17, 21].

Another novel finding reveals a homeostatic loop where Sorcin is overexpressed in PTX-resistant cancer cells involved in cell proliferation, EMT, stemness, and PTX-resistance. Mechanistically, miR-142-5p binds to the 3′-UTR of SRI and dampen its expression. On the contrary, ZEB1 inhibits the miR-142-5p transcription by obstructing its promotor E-box binding sites. ZEB1 is, in turn, negatively regulated by Sorcin, which momentarily interacts with the Smad4 complex to block its translocation from the cytosol to the nucleus. Therefore, targeting this SRI/Smad4/ZEB1/miR-142-5p loop may reverse the PTX-resistance and prevent the malignant progression [85].

TGF-β1 and TNF-α inhibit sorcin expression

Sorcin overexpression was linked to impaired TGF-β signaling. Treatment with TGF-β1, inhibit Sorcin, suggesting that restoring calcium homeostasis by reducing Sorcin expression can reverse chemoresistance. Notably, TGF-β1 inhibits Sorcin expression, further highlighting its relevance in cancer progression [4]. Additionally, TNF-α downregulates Sorcin in pancreatic cells by increasing the phosphorylation of key signaling pathway, such as mTOR and NF-κB, while

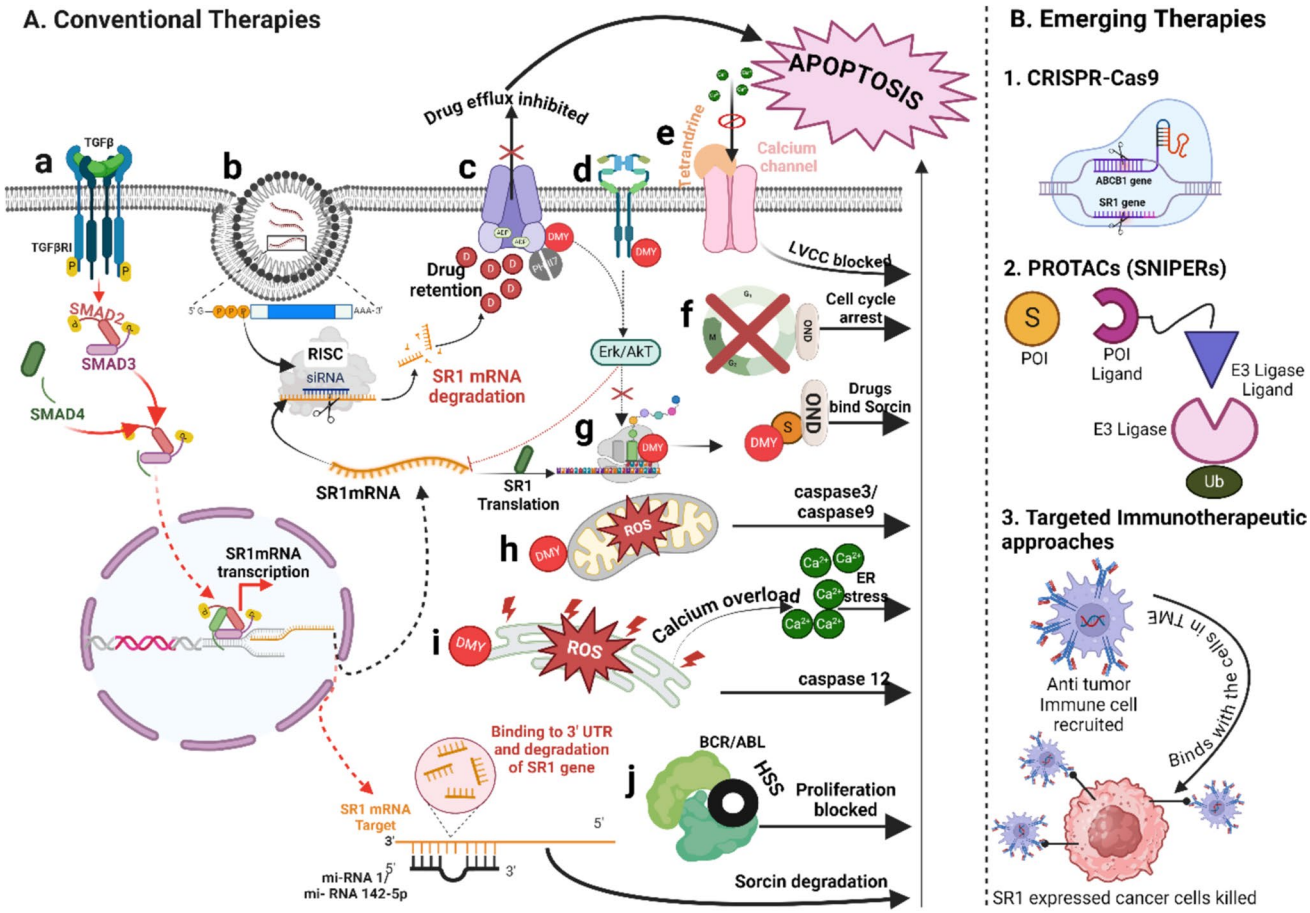

Fig. 4 Overview of conventional and emerging therapeutic approaches targeting Sorcin for revising chemoresistance in Cancer: (A) **a**. Conventional therapies highlighting mechanisms of action such as miR-1/SRI and SRI/Smad4/ZEB1/miR-142-5p signaling for SR1 degradation leading to apoptosis. **b**. LANP-PTX-siSRI nanocarrier targeting Sorcin degradation. **c**–**i**. Phytochemicals and small molecules inhibiting Sorcin and inducing apoptosis through different pathways B. Emerging therapies showcasing innovative strategies including CRISPR-Cas9 gene editing, PROTACs for targeted protein degradation, and CAR-T cell therapy for enhanced immune response against cancer cells

fractalkine (CX3CL1) recovers Sorcin levels by dampening mTOR expression and phosphorylation of both mTOR and NF-κB. This highlights its involvement in cancer progression and its relevance in perturbed TGF-β signaling [86].

Phytochemical and small molecule targeted sorcin silencing

Conventional mono or combinational therapeutic agents known to upregulate the expression of MDR regulator Sorcin, which enhances resistance and reduces therapeutic efficacy in cancer treatments. A recent study investigating dihydromyricetin (DMY), and ondansetron (OND) co-administration observed that DMY effectively inhibited the Sorcin expression whereas OND did not. This inhibition subsequently suppressed p-ERK, and p-Akt expression thereby reducing P-gP and mitigate ADR-induced G2/M cell cycle arrest [49]. Additionally, in a randomized controlled trial, Haishengsu (HSS), a marine-derived drug, was found to improve the quality of life of the drug-resistant leukemia patients. HSS suppresses the expression of Sorcin in a dose depended manner among Adriamycin resistant leukemia patients thus promoting drug sensitivity [87]. Furthermore, Tetrandrine (Tet) enhance Sorcin level at lower concentration but suppresses at higher concentration contributing to reversal of drug resistance [88]. PH II-7 is a potent inhibitor of multidrug-resistant tumor cells. PH II-7 inhibits the expressions of Sorcin, increase the intracellular drug concentration and induce apoptosis [89]. Palmitate reduces Sorcin expression at mRNA level in normal HEK293 cells, reduces ATF6 activity despite increasing ER stress. However, the precise signaling cascade resulting this effect of palmitate on Sorcin expression is unclear, but could likely include palmitoylation, TNFα, NFκB or Toll-like receptor 4 signaling [43]. Additionally, Calcitriol treatment found to increase the Sorcin expression, however, its regulation and consequence

of drug reversal needs further exploration [90]. Palmitate, downregulates Sorcin expression in pancreatic beta cells, contributing to lipotoxicity and endoplasmic reticulum (ER) stress, through AMPK phosphorylation while upregulating pro-apoptotic markers such as pEIF2α, CHOP, and cleaved caspase-3. Interestingly, metformin counteracts these effects by restoring pAMPK, CHOP, pEIF2α, and cleaved caspase-3 levels, while simultaneously enhancing Sorcin expression [91, 92].

Although current Sorcin-directed existing therapies have shown potential in some experimental cancer models, the complexity of MDR cancers requires advanced innovative strategies to overcome the limitations of present-day treatments. The following texts discuss several promising emerging therapies aimed at overcoming MDR that may involve targeting Sorcin.

Emerging MDR therapies with potential to target sorcin in future

Although technologies like RNAi, nanotechnology have been employed to target Sorcin, further advancements in these and other technologies are needed to allow precise genetic editing such as CRISPR-Cas9, to effectively target Sorcin, mitigating its role in cancer cell survival and chemotherapy resistance. Additionally, several novel therapeutic agents are in clinical trials suggest a paradigm shift in the approach such as liposomes, targeted drug carriers, Immunotherapy, including checkpoint inhibitors, cancer vaccines, and CAR-T cell treatment might selectively target and eradicate Sorcin expression in cancer (Fig. 4B).

ABC transporters such as P-glycoprotein (ABCB1/MDR1), ABCG2 (BCRP) and MRP-1 (ABCC1) are often overexpressed after the initial treatment regimen and are unresponsive to certain chemotherapeutics drugs. Current therapeutics involve CRISPR-Cas9-mediated ABCB1 knockout to reverse MDR in improving long-term treatment efficacy [93]. Moreover, EMT is concerned with cancer metastasis and drug resistance, targeting EMT may have a therapeutic gain [61]. Around 20–24 nucleotide-miRNAs are involved in connecting EMT and ABC transporters. miR-200c and miR-145 inhibit ABC transporters and EMT markers by binding at 3′-UTR and gene promoter region. MiRNAs can control the level of ABC transporters [94]. In accordance with the above studies, Sorcin can also be good target for CRSIPR- based strategy, by allowing the gene-editing technology to manipulate the specific SR1 gene and may rectify the mutations. Targeted protein degradation has appeared as a potential approach to combat drug resistance in cancer. This strategy uses small molecules like PROTACs and SNIPERs. PROTACs recruit E3 ubiquitin ligase for protein degradation even non-enzymatic proteins rendering the development of drug resistance, for its degradation by the ubiquitin–proteasome system. For instance, PROTACs targeting BCR-ABL1, EGFR, androgen receptor has shown to circumvent the drug resistance mechanisms restoring chemosensitivity of the cancer cells. Whereas, SNIPERs employ the cellular IAPs to facilitate the degradation of target proteins. SNIPERs induce degradation independent of ubiquitin–proteasome pathway through alterations in this pathway [95]. Similarly, Sorcin can act as protein of interest (POI) as it displays three binding pockets for calcium binding that can be exploited for PROTACs designing and targeted for protein ubiquitination. Tumor microenvironment plays a crucial role in facilitating interactions between cancer cells and non-malignant cells, leading to inhibition of immune clearance of tumor cells, metabolic reprogramming, induction of soluble paracrine factor release, initiation of changes in peri-tumor stomatal cells and blood vessels and promote drug resistance. Recently, targeting tumor microenvironment in the treatment of drug resistance cancers has demonstrated the positive therapeutic effects offering potential avenues for reversing drug resistance or relapsed tumors. The development of drugs that remain active under hypoxic conditions and the inhibition of MDR proteins are imperative [96]. A recent study has revealed Sorcin overexpressing tumor cells forms positive feedback loop STAT3 which plays an important role in immune cells maturation and regulating inflammation by modulating inflammatory cytokines in pancreatic cells (Ref: Gong, Jiali, et al. *bioRxiv* (2023): 2023–07). Some potential treatment strategies can be anti-JAK/STAT monoclonal antibody, inflammatory cytokines inhibitors, cytokine receptor antagonists may modulate the inflammatory TME to achieve targeted therapy.

Taken all together, by disrupting its function, these advanced strategies could impede the role of Sorcin in drug-resistant cancers, offering a new pathway to overcome the limitations of conventional treatments and improve patient outcomes. Additionally, the effectiveness of these strategies in clinical settings has to be fully demonstrated. Although preclinical studies may show promise, the translation of these findings into effective treatments requires rigorous testing in diverse patient populations.

Clinical inference of sorcin

Patient models are vital for elucidating the clinical relevance of Sorcin and predicting therapeutic responses applicable for clinical trials. Sorcin is prevalent among cancer patients and at transcriptional level, it demonstrates a latent association with clinicopathological and survival outcomes, highlighting its clinical significance and prognostic importance [41]. Furthermore, genomic alterations in Sorcin have been profiled in a limited number of patients across distinct cancer types,

with amplification being the most common alteration succeeding deletion, missense, truncating mutations and splice variants. On the contrary, missense mutation predominate in uterine carcinomas and SRI was significantly more frequent in patients receiving neoadjuvant chemotherapy prior to resection. Meanwhile, deletions are notably prevalent in metastatic breast cancer [11] [101]. A pan-cancer study has observed a substantial decrease in Sorcin expression from stage I through stage IV carcinomas. However, in other cancers, Sorcin expression reflects a positive connotation with older age groups, tumor status (advanced pathological and clinical stage), local invasions, lymph node metastasis and overall survival in some cancers. Moreover, SRI expression was analyzed in relation to cox proportional hazards model and the Kaplan–Meier analysis. The results revealed a significant correlation between Sorcin and overall survival in different cancers, acting as either a high- or low-risk factor depending on the cancer type. high SRI levels predicted poor or short overall survival, adverse factor for progression-free survival, and poor disease-free survival [97]. Additionally, Sorcin is inversely correlated with chemotherapy response of patients and overall prognosis [33, 98]. Correspondingly, Sorcin has been identified in the serum of patients who are complete responders, partial responders and those with stable disease following chemotherapy. This evidence suggests the involvement of Sorcin in the development of drug resistance among complete and partial responder breast cancer patients [18].

Conclusion and the future perspective

Sorcin has not been explored widely in context of various cellular processes involved in cancer; however, its impact on cancer hallmarks and drug resistance underlines its therapeutic potential. Looking ahead, its understanding of the elaborate and concrete molecular mechanisms governing different aspects of these hallmarks remains inadequate. Emerging evidence concerning its presence in distinct cell types, tumor tissues and cancer-associated serum foresees the necessity of investigating Sorcin in greater detail and clarity. Similarly, research on Sorcin, envisioned significant contributions in understanding of multidrug resistance and its interaction with different therapeutic drugs. However, the discrete mechanism regulating its role in drug resistance is yet to be resolved. Currently, several anticancer drugs, RNAi, have been discovered to target Sorcin at the transcriptional and translational levels in different cancer drug resistance models. An in-depth understanding of Sorcin, including its fundamental mechanisms, could carve the way for the discovery and development of novel drugs targeting Sorcin thereby benefiting bench-to-clinic approaches in cancer treatment. The insight provides an attractive avenue for precision medicine, targeted therapies, and drug development. Future research should focus on mapping its concrete role across different cancers, clarifying its influence on various signaling pathways, and optimizing targeted therapies.

Acknowledgements Conception and design: SG and VDN. Data collection and manuscript drafting: SG. Figure creation: AS and SG. Critical review and editing: VDN, RSK and AS. All authors contributed to the review article and approved the submitted version.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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Dear Dr. Vilas D Nasare

The APJCP editorial team is glad to inform you that your manuscript titled **"*Comparative study of ERK1/2, Bcl2, and Sorcin with Clinicopathological Characteristics of Gastric carcinoma: Tubular Adenocarcinoma Vs Signet Ring Cell Carcinoma*"** has been accepted for publication and will be scheduled for publication as soon as we receive the documentary for processing fee payment.

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Effect of overexpression of ERK1/2 and MDR1 on chemoresistance in advanced gastric cancer patients.

Authors: Sushmita Ghosh, Ranita Pal, Tanuma Mistry, Pranab Kumar Sahoo, Sutapa Mahata, Sinjini Sarkar, Trisha Choudhury, ... SHOW ALL ..., and Vilas Nasare | AUTHORS INFO & AFFILIATIONS

Publication: Journal of Clinical Oncology • Volume 41, Number 16_suppl • https://doi.org/10.1200/JCO.2023.41.16_suppl.e16051

Abstract

e16051

Background: Gastric cancer (GC) is late diagnosed disease with a poor prognosis. The emergence of multidrug resistance (MDR) increases the disease burden, and treatment failure in the patients. Several molecular and circulating biomarkers have been identified and evaluated for the clinical relevance in the management of gastric cancer. The aim of this study is to decipher the involvement of ERK1/2 and MDR1 in relation to MDR phenotype and major clinicopathological characteristics in gastric cancer patients. This will help to select effective drug combinations to improve the treatment success rate. **Methods:** This study recruited 64 individuals with newly diagnosed gastric cancer between 2019 and 2022. Patients were recommended for surgery or neoadjuvant chemotherapy (NACT). NACT group treated with docetaxel (60 mg/m2), oxaliplatin (85 mg/m2), leucovorin (200 mg/m2), and 5-fluorouracil (2,600 mg/m2 as a 24 hr infusion), all given on day 1 and administered every 2 weeks before surgery. Based on CECT report, patients were categorized as responders (CRs), partial responders (PRs), and non-responders (NRs). Tissue and blood samples were analyzed for ERK1/2 and MDR1expression using IHC, immuno-blotting and qRT-PCR analyses. Rhodamine123 (Rh123) accumulation assay and flowcytometry was performed to measure the degree of resistance among patients. Descriptive statistics, and Pearson's Chi-square test was carried out to evaluate the expression distribution of ERK1/2 and MDR1. **Results:** Out of 64 patients, between the ages of 41-60 (53.08±10.51), 54.5% have ≥5 cm tumor size in the antrum site (56.1%) and diagnosed with stage III (56.1%). Majority of observations were intestinal type (60.6%) with lymphovascular invasion (71.2%). Among all 29.4%, 20.6% and 50.0% were CRs, PRs, & NRs respectively and 36.5% patients underwent NACT. The IHC scoring analysis revealed 25 (37.9%) & 18 (28.6%) individuals for ERK1/2 and 18 (27.3%) and 26 (39.4%) individuals for MDR1 had moderate to strong expressions respectively. Simultaneously, the expressions were highly significant with tumor site (antrum), tumor size (≥5 cm), wall thickening (radiological finding), signet ring cell carcinoma, grade 3, stage III (T3N2), NRs, lymphovascular, peritoneal and serosal invasions (p=0.000). ERK1/2 was more prevalent in diffuse type patients (p=0.000). Also, protein and mRNA level for both genes were more pronounced in NACT group (p<0.005). Flowcytometry showed FLOT receivers had lower Rh123 accumulation, indicating MDR1overexpression (11.5± 8.9) compared to baseline (49.5 ±38.3).

likelihood of reaching cycle 3, which could imply better outcomes. PFS was slightly higher and consistent with previously reported studies.

Editorial acknowledgement: Authors thanks Juan Luis Sanz (APICES) for his editorial assistance.

Legal entity responsible for the study: The authors.

Funding: Bayer.

Disclosure: R. Afonso: Advisory / Consultancy: Servier, Bayer. C. López-López: Advisory / Consultancy: Merck, Amgen, Roche, Bayer, Servier; Speaker Bureau / Expert testimony: Merck, Roche, Servier; Research grant / Funding (self): Merck, Amgen, Roche, MSD, Servier; Travel / Accommodation / Expenses: Merck, Amgen, Roche, Servier, MSD. J. Reina Zoilo: Advisory / Consultancy: Bayer, Servier. All other authors have declared no conflicts of interest.

https://doi.org/10.1016/j.annonc.2023.04.059

P-4 KRAS mutation and survival of rectal cancer in Korea between 2010 and 2015: Analysis of the Collaborative Stage Data of Korean Central Cancer Registry

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Background: KRAS mutation status is known to have prognostic value in patients with colorectal cancer. However, prognostic value of KRAS mutation is not well established in patients with rectal cancer alone. This study aimed to evaluate survival of overall rectal cancer patients according to KRAS mutation status.KRAS mutation status is known to have prognostic value in patients with colorectal cancer. However, prognostic value of KRAS mutation is not well established in patients with rectal cancer alone. This study aimed to evaluate survival of overall rectal cancer patients according to KRAS mutation status.

Methods: The Korean Center of Cancer Registry performed the Collaborative Stage Data Survey between 2010 and 2015 to establish collaborative stage data (CSD) of cancers of stomach, colon, rectum, rectosigmoid junction, and breast. Information on patients with rectal cancer was extracted from the CSD for analysis. Variables including age, sex, diagnosis date, American Joint Committee on Cancer (AJCC) staging system scores were analyzed. Cox proportional hazard regression analyses were used to evaluate prognostic factors and survival analysis was performed using the Kaplan—Meier method.

Results: A total of 1549 rectal cancer patients with KRAS test result were identified, among them 990 (63.9%) had wild type KRAS and 559 (36.1%) had mutated KRAS. Multivariate analysis showed that mutated type KRAS was an independent risk factor for survival (Hazard ratio (HR): 1.267, 95% confidence interval (CI): 1.052-1.526). Overall survival of wild type KRAS was significantly higher than that of mutated type KRAS (p=0.007). When stratified according to AJCC7 stages, survival curves of wild type KRAS showed higher survival to that of mutated type KRAS in AJCC7 stage II-III (p=0.023).

Conclusions: Mutated KRAS status had significantly worse prognostic value in patients with rectal cancer, especially in stage II-III.

Legal entity responsible for the study: The authors.

Funding: Has not received any funding.

Disclosure: All authors have declared no conflicts of interest.

https://doi.org/10.1016/j.annonc.2023.04.060

P-5 A soluble resistance related calcium binding protein (sorcin) up-regulates in the multi-drug resistant advanced gastric cancer patients

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Background: One of the major challenges to neoadjuvant chemotherapy (NACT) for gastric cancer is acquired multidrug resistance (MDR). The MDR cells in the heterogenous tumor usually survive the chemotherapy and leads to disease progression and relapse. Identification of potential biomarkers to predict the MDR phenotype prior chemotherapy and to develop strategies for new drug combinations is crucial. Sorcin, a newly recognized resistance-related protein, has been reported to up-regulate in many drug-resistant cancers. This investigation intends to detect sorcin status in MDR patients to the FLOT regimen in locally advanced gastric cancer patients.

Methods: Based on inclusion criteria, 63 participants who were diagnosed with advanced GC at the Chittaranjan National Cancer Hospital were enrolled in this study. The patients were categorized based on treatment modality, pre-NACT (Surgery first) and post-NACT. Post-NACT group treated with 5-fluorouracil (2,600 mg/m2 as a 24 hr infusion), leucovorin (200 mg/ m^2), oxaliplatin (85 mg/ m^2), and docetaxel (60 mg/ m^2), all given on the same day and administered every 2 weeks before surgery. According to the CECT report, responders, partial responders and complete responders were assessed. Tissue samples were collected after surgery from both the categories and subjected to IHC, immunoblotting and qRT-PCR analysis to detect the expression level of sorcin. The correlation between sorcin and clinicopathological characteristics was determined by descriptive statistics using the Pearson $\chi 2$ test.

Results: Out of 63, 23 (36.5%) patients underwent NACT. 44 (69.8%) were males, mostly age between 41-60 (53.33 $\pm$10.4) years. The lymphovascular invasion was present in 71.2% of the GC patients. When compared to normal adjacent tissue, sorcin expression was higher (H-score 3) in 36 (54.5%) patients and in the patients with tumor sizes $\geq$5 cm (24 (66.6%)). Sorcin level was also significant with post-NACT (69.5%) cases (p=0.05), lymphovascular invasion (p=0.026), tumor stage (T3; p=0.000), lymph node involvement (p=0.018) and pTNM staging (stage IIIA & IIIB; p=0.004). The partial responders and non-responders showed moderate to high sorcin expression in both the categories. However sorcin expression had significant difference between the responders and non-responders among the post-NACT group (p=0.0008). Immunoblotting and qRT-PCR further confirmed the similar expression pattern. When compared to responders, both partial responders and non-responders had a >7-fold increase of sorcin expression ($p < 0.05$) in post-NACT group. The mRNA analysis also revealed higher mean ΔCT for non-responders (9.7$\pm$0.10).

Conclusions: Sorcin levels remain moderate to high in non-responders. Further, post-NACT group treated with FLOT identified to enhance the sorcin expression among the non-responders. Therefore, sorcin can be a potential biomarker to diagnose the multidrug resistance phenotype in advanced gastric cancer patients.

Legal entity responsible for the study: Vilas D Nasare.

Funding: Funded by Chittaranjan National Cancer Institute, Kolkata, India, received to Sushmita Ghosh.

Disclosure: S. Sarkar: Research grant / Funding (self): Department of Health Research, Govt of India. All other authors have declared no conflicts of interest.

https://doi.org/10.1016/j.annonc.2023.04.061

P-6 Postoperative anastomotic leakage following minimally invasive esophagectomy for esophageal carcinoma: A single center retrospective analysis

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Background: Esophageal cancer is a common malignancy among the Asian population. Minimally invasive esophagectomy(MIE) is rapidly gaining popularity in the management of these patients due to the benefits of faster postoperative recovery and less pulmonary complications. However anastomotic leakage remains a potential postoperative complication with great impact on clinical outcome after esophagectomy.

Methods: Between 2016 to 2022, 75 patients with esophageal carcinoma underwent MIE in our institution. All patients had a routine water-soluble contrast study on postoperative day 5 to assess anastomotic integrity. The clinicopathologic characteristics, postoperative outcomes and management of patients who developed postoperative anastomotic leakage were evaluated.

Results: Thirteen (17.3%) patients developed postoperative anastomotic leak in this study population. All patients were males with a mean age of 66yr (range, 57-76). Nine patients had cardiovascular risk factors and 10 were smokers. Mean BMI was 21.4. Tumors were located in the mid esophagus(4 patients), lower esophagus(5) and cardio-esophageal junction(4). Tumor histology — Squamous cell carcinoma in 4 patients and Adenocarcinoma in 9. Out of the 13 patients, 8 patients were following neoadjuvant therapy and 2 patients were operated following recurrence after previous curative therapy. Except for one patient all others had R0 resection on final histological assessment. Nine patients developed anastomotic leak following McKeowns three-stage MIE, while 4 patients were following Ivor Lewis two-stage MIE. Presentation ranged from asymptomatic(radiologically detected) to lung infections with pleural/mediastinal collections to severe mediastinitis. Three patients with cervical leaks were successfully managed conservatively with intravenous antibiotics, wound drainage and supportive care. Two other patients with cervical leaks were managed with internal drainage by endoscopic placement of double pigtail stent. Two physiologically stable patients with contained anastomotic leakage were managed with internal drainage (endoscopic placement of double pigtail stents) along with image guided pleural drain insertion. Others required a combination of treatment modalities including surgical intervention. Initial step included video assisted thoracoscopy(VATs) and on-table upper GI endoscopy (OGD) to assess degree of anastomotic dehiscence and pleural cavity washout. Repair of the anastomotic defect was successfully attempted in one patient with favorable conditions. Others required exteriorization of the defect by tube drainage. In addition endoscopic covered esophageal stents were used in one patient. Naso-jejunal tubes and feeding jejunostomy were utilized to maintain enteral nutrition. Only one patient required take down of the gastric conduit and cervical esophagostomy. These patients had an extended hospital stay, mean 50 days (range,16-124). Two mortalities due to anastomotic leakage were noted (2.6%).

Conclusions: Patients with leaks from thoracic anastomoses required aggressive intervention compared to cervical anastomosis leakage. Extend of anastomotic