AN INHIBITOR OF THE MITOTIC KINASE, MPS1, IS SELECTIVE TOWARDS PANCREATIC CANCER CELLS

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The abysmal five year pancreatic cancer survival rate of less than 6% highlights the need for new treatments for this deadly malignancy. Cytotoxic drugs normally target rapidly dividing cancer cells but unfortunately often target stem cells resulting in toxicity. This warrants the development of compounds that selectively target tumor cells. An inhibitor of the mitotic kinase, MPS1, which has been shown to be more selective towards cancer cells than non-tumorigenic cells, shows promise but its effects on stem cells has not been investigated. MPS1 is an essential component of the Spindle Assembly Checkpoint and is proposed to be up-regulated in cancer cells to maintain chromosomal segregation errors within survivable limits. Inhibition of MPS1 kinase causes cancer cell death accompanied by massive aneuploidy. Our studies demonstrate that human adipose stem cells (ASCs) and can tolerate higher levels of a small molecule MPS1 inhibitor than pancreatic cancer cells. In contrast to PANC-1 cancer cells, ASCs and telomerase-immortalized pancreatic ductal epithelial cells did not exhibit elevated chromosome mis-segregation after treatment with the MPS1 inhibitor for 72hrs. In contrast, PANC-1 pancreatic cancer cells exhibited a large increase in chromosomal mis-segregation under similar conditions. Furthermore, growth of ASCs was minimally affected post treatment whereas PANC-1 cells were severely growth impaired suggesting a favorable therapeutic index. Our studies, demonstrate that MPS1 inhibition is selective towards pancreatic cancer cells and

that stem cells are less affected *in vitro*. These data suggest MPS1 inhibition should be further investigated as a new treatment approach in pancreatic cancer.

Brenda R. Grimes, Ph.D., Chair

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LIST OF ABBREVIATIONS

%MD	Percent Modal Deviation
µmol/L	micromoles/litre
APC/C	Anaphase Promoting Complex
ASC	Adipose derived mesenchymal stem cells
ATP	Adenosine triphosphate
ATCC	American Type Culture Collection
BME	b-Mercaptoethanol
BRCA1	Breast Cancer 1
BSA	Bovine Serum Albumin
cdc20	Cell-division cycle protein 20
CDK1	Cyclin-dependent kinase 1
CIN	Chromosomal Instability
CGH	Comparative Genomic Hybridization
DNA	Deoxyribonucleic acid
DAPI	6-diamidino-2-phenylindole
DMSO	Dimethyl Sulfoxide
DSB	Double Strand Break
ECL	Enhanced Chemilumescent
EDTA	Ethylenediaminetetraacetic acid
EGM2-MV	Microvascular Endothelial Cell Growth Medium-2
FBS	Fetal Bovine Serum
FISH	Fluorescence *In Situ* Hybridization

G1	Gap1
G2	Gap2
HER2	Human Epidermal Growth Factor 2
hTERT-HPNE	Human telomerase reverse transcriptase immortalized pancreatic ductal cells
IC_{50}	Half maximal inhibitory concentration
KCl	Potassium Chloride
KRAS	Kirsten rat sarcoma viral oncogene homolog
KRC	*Kras* combined with *Rb* (Retinoblastoma) gene deletion using *Cre*-recombinase
LOH	Loss of Heterozygosity
LSI	Large-scale Integration
MAD1	Mitotic spindle assembly checkpoint protein
MAD2	Mitotic arrest deficient 2
MAP	Microtubule Associated Proteins
MCC	Mitotic Checkpoint Complex
MPS1	Monopolar Spindle 1
MT	Microtubule
MTOC	Microtubule Organizing Center
nCIN	Numerical Chromosomal Instability
nmol/L	nanomoles/litre
noc	Nocodazole
PAGE	Polyacrylamide Gel Electrophoresis

PARP	Poly ADP ribose polymerase
PBS	Phosphate Buffered Saline
PBS-T	Phosphate Buffered Saline-Tween
PDAC	Pancreatic Ductal Adenocarcinoma
pS10H3	phosphorylated histone H3 serine 10
PVDF	Polyvinylidene Fluoride
RB	Retinoblastoma
RIPA	Radioimmunoprecipitation assay
rpm	Revolutions Per Minute
RPMI	Roswell Park Memorial Institute Cell Culture Medium
SCC1	Sister chromatid cohesion protein 1
sCIN	Structural Chromosomal Instability
SAC	Spindle Assembly Checkpoint
SDS	Sodium Dodecyl Sulfate
SKY	Spectral Karyotype
SNP	Single Nucleotide Polymorphism
SSC	Saline-sodium citrate
TNBC	Triple-Negative Breast Cancer
TTK	Receptor Type Tyrosine Kinase
v/v	Volume per volume
w/v	Weight per volume

INTRODUCTION

1. Mitosis

Mitosis is the process of cell division of the somatic cells of eukaryotic organisms. The process is essential for the development, maintenance and regeneration of an individual. It is a part of the cell cycle which consists of four phases namely G1 (Gap1), S (DNA Synthesis), G2 (Gap2) and M (Mitosis) phase. Mitosis is divided in six stages such as prophase, prometaphase, metaphase, anaphase, telophase and cytokinesis. During prophase, the DNA is coiled and supercoiled to form compact genetic structures called chromosomes and the spindle apparatus begins to form followed by the disappearance of the nuclear membrane in prometaphase. Also, chromosomes are aligned at the metaphase plate, which is the cell's equator, throughout metaphase after which the sister chromatids begin their journey to the opposite sides of the cell in anaphase. Cell division is completed with the process of cytokinesis where the mother cell is divided into two daughter cells. The mitotic spindle apparatus ensures that the daughter cells receive equal amount of DNA and it consists of the centrosomes, kinetochore, microtubules (MT) and microtubule associated proteins (MAPs) [Figure 1 adapted from (Bruce Alberts 2002)]. The centrosomes establish MT polarity and determine the number and distribution of microtubules. Each centrosome is composed of two centrioles, which are open-ended cylinders, each comprising of nine sets of triplet MTs linked together, plus some surrounding pericentriolar material. The kinetochore is a protein structure on chromatids where the spindle fibers attach during cell division to pull sister chromatids apart. The

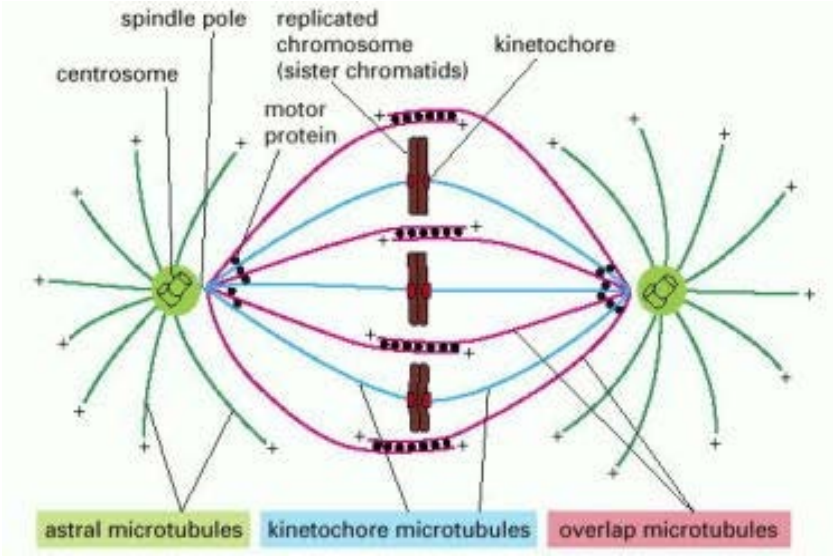

Figure 1 Schematic representation of the spindle apparatus in metaphase

Chromosomes are aligned at the metaphase plate. Microtubules arising from the centrosome attach to the kinetochores on each sister chromatid. The plus ends (growing ends) are away from the spindle poles. Spindle microtubules are classified into three categories:

1. Astral microtubules that radiate in all directions, contribute to forces that separate the poles and are responsible for orientation and positioning of the spindle
2. Kinetochore microtubules which attach to the kinetochore
3. Overlap Microtubules that interdigitate at the equator of the spindle and are responsible for the symmetrical and bipolar shape of the spindle

[Figure adapted from (Bruce Alberts 2002)]

kinetochore contains open chromatin but it assembles within a largely heterochromatic domain which is located on the centromere and is made of non-coding sequences called the repetitive alpha satellite DNA (Figure 2). The microtubules are the fundamental machinery of the spindle apparatus originating in the microtubule organizing center (MTOC), which is a part of the centrosome. These microtubules are hollow cylinders that are formed by the polymerization of a dimer of two globular proteins, alpha and beta tubulin and they form the spindle fibers that attach to the kinetochore and pull the sister chromatids away from each other towards the opposite ends of the cell. The attachment of the kinetochore and microtubules is governed by the spindle assembly checkpoint complex (SAC) which will be described in detail in later sections. Errors at any stage of the cell division lead to an abnormal number of chromosomes in daughter cells and/or structurally aberrant chromosomes causing genetic instability.

2. Chromosomal Instability

2.1.Definition of chromosomal instability

Chromosomal instability (CIN), a form of genomic instability, is defined as an increased rate of change of number and/or structure of chromosomes. It was first defined by Vogelstein and his team in 1997 as ‘a striking defect in chromosome segregation, resulting in gains or losses in excess of 10^{-2} per chromosome per generation’ (Lengauer, Kinzler, and Vogelstein 1997). Since then, CIN has been shown to be promoted by many different mechanisms including defects in chromosome segregation, disturbances in the

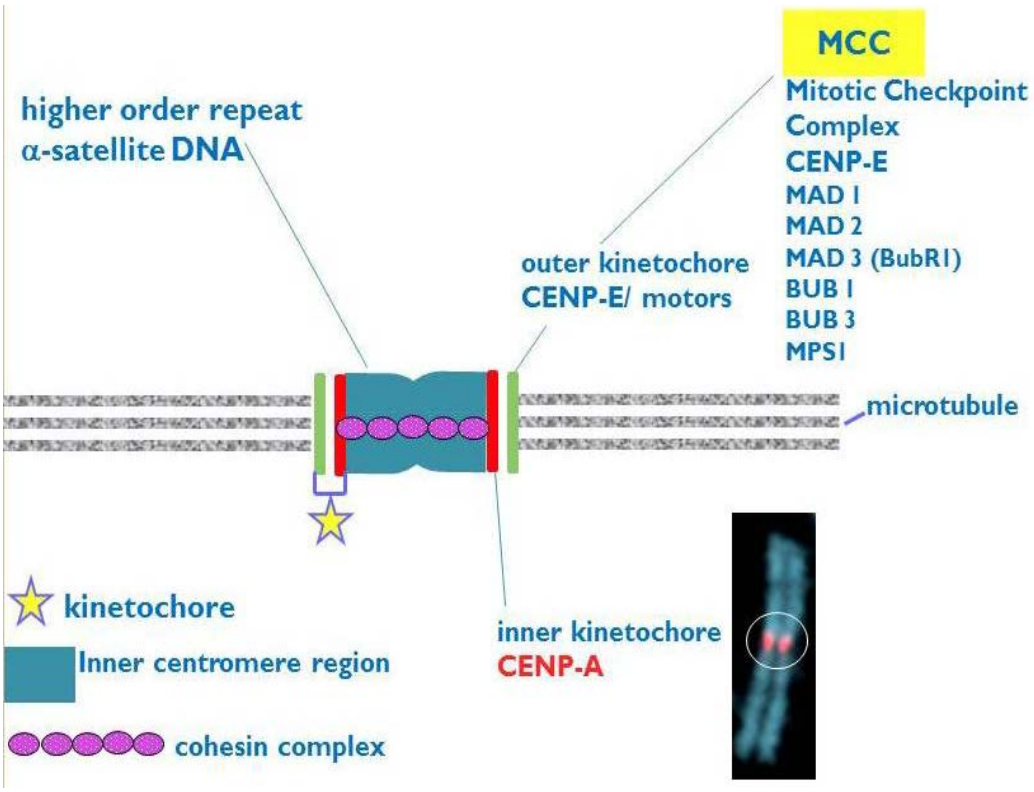

Figure 2 Schematic representation of the organization of the kinetochore at the centromere

The centromere consists of a higher order repeat of α-satellite DNA on which the kinetochore is built during mitosis. The sister chromatids are held together by cohesin complex until the end of metaphase. The inner kinetochore is marked with the epigenetic marker CENP-A, which is a variant of histone H3. The outer kinetochore is in contact with a kinesin motor protein called CENP-E and the proteins from the mitotic checkpoint complex also called the spindle assembly checkpoint (SAC).

[Figure courtesy: Dr. Brenda Grimes]

cell cycle and faulty DNA damage repair systems (Thompson, Bakhoum, and Compton 2010). Mutations in tumor suppressor genes such as p53 and RB also lead to cells becoming chromosomally unstable (Gordon, Resio, and Pellman 2012). Figure 3 summarizes the four primary defects in mitosis that give rise to CIN namely, impaired SAC, defects in the geometry of the centromere, faulty attachments between the kinetochore and the microtubule and changes in the number of centrosomes in a cell [Figure 3 adapted from (Orr and Compton 2013)]. The activity or expression of the proteins that regulate these mechanisms are affected by mutations and changes in their upstream factors. For example, loss of function of the tumor suppressor gene RB disrupts the localization of cohesin proteins on the centromere thereby altering sister chromatid cohesion during cell division and causing CIN (Orr and Compton 2013, Manning, Longworth, and Dyson 2010). CIN and aneuploidy are interrelated, but both are distinguished from each other as CIN being the ‘rate of chromosomal mis-segregation’ and aneuploidy being the ‘state of abnormal chromosome number’ (Bakhoum and Compton 2012).

CIN is classified into (1) numerical CIN (nCIN), which results in deviation of the normal chromosome number resulting in aneuploidy, and (2) structural CIN (sCIN), reflecting changes in the structure of the chromosome via mechanisms such as translocations, deletions, amplifications and inversions [Figure 4 adapted from (McGranahan et al. 2012)]. nCIN is caused by different mechanisms such as errors in mitotic checkpoints, defects in the assembly of the spindle apparatus, defects in sister chromatid cohesion, amplification of centrosomes and faulty attachment of spindle microtubules to the

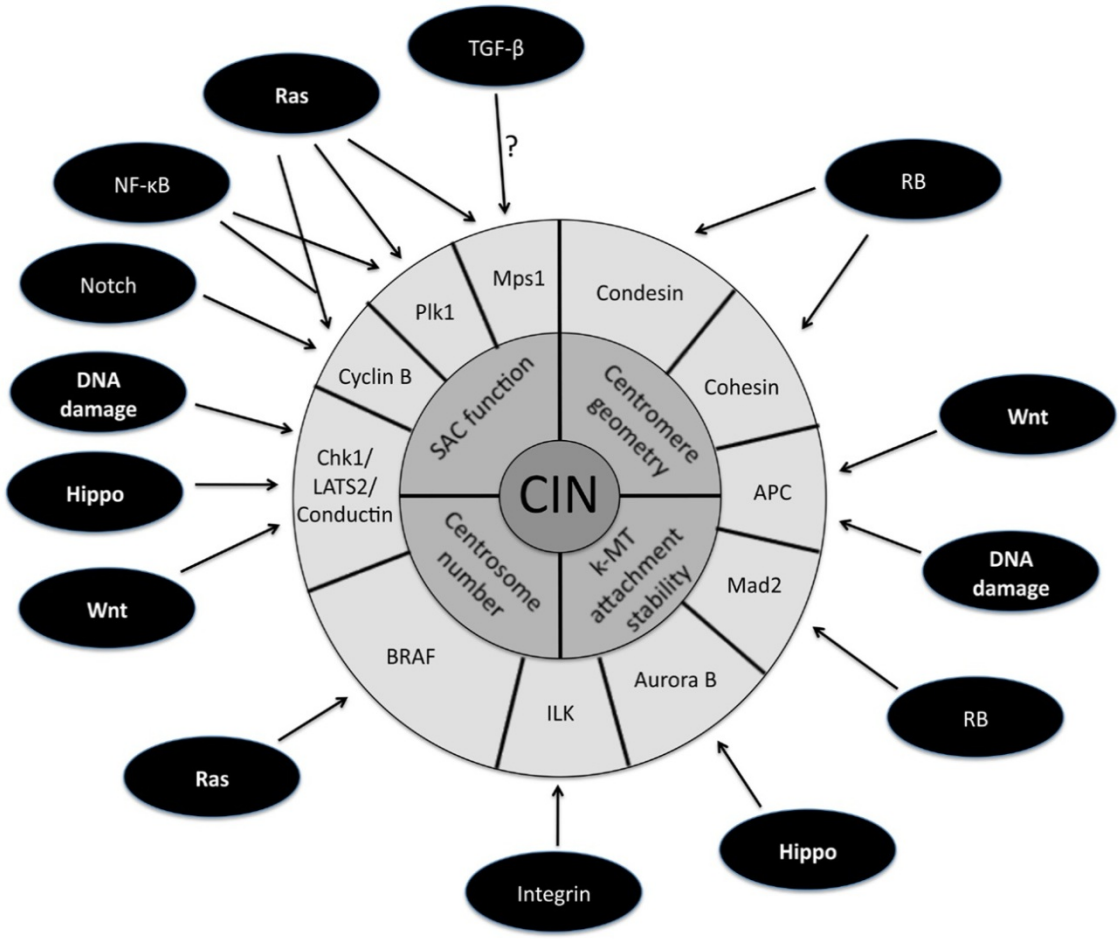

Figure 3 Schematic representation of mitotic defects that cause CIN

The innermost circle represents chromosomal instability (CIN). The middle circle comprises the four main defects in mitosis that are known to cause CIN while the outermost circle depicts the factors that regulate these mechanisms. These factors are the downstream targets for various oncogenic pathways shown in the bubbles.

[Figure adapted from (Orr and Compton 2013)]

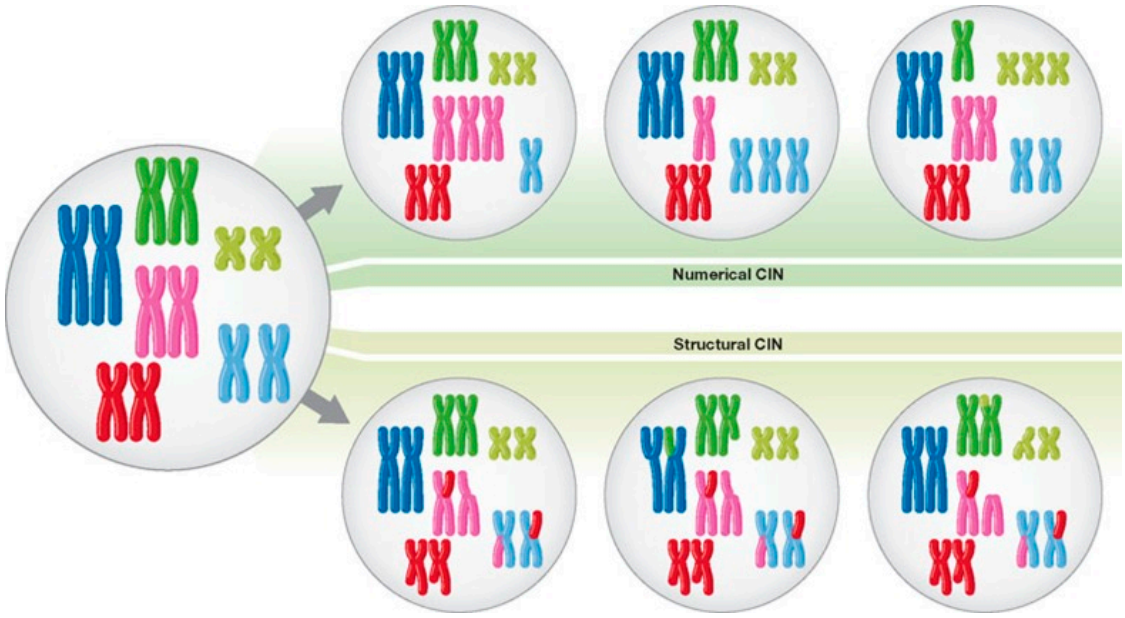

Figure 4 Schematic representation of numerical and structural chromosomal instability

Numerical chromosomal instability (nCIN) is characterized by gains and losses of whole chromosome(s) whereas structural chromosomal instability displays structural rearrangements such as translocations, deletions and amplifications within and between arms of chromosomes.

[Figure adapted from (McGranahan et al. 2012)]

kinetochores (McGranahan et al. 2012). It is also reported that DNA damage occurs during aberrant chromosome segregation which can result in the subsequent structural rearrangement of chromosomes and contribute to sCIN (Janssen et al. 2011, Crasta et al. 2012). sCIN is also reported to be associated with 'reactive' chromosomes which are formed after chromosome breaks. These reactive chromosomes give rise to 'Breakage-Fusion-Break' cycles which in turn cause genomic rearrangements (Gisselsson et al. 2000). Both nCIN and sCIN are often correlated with each other in cancer cells and result in a complex karyotype (Roschke et al. 2003, Gisselsson et al. 2000).

CIN can have profound effects on the cell. The presence of CIN may lead to the formation of fusion gene products as well as deletion and amplification of genes making which in turn may propagate CIN further (McGranahan et al. 2012). Clinically, CIN causes congenital abnormalities, anatomic malfunctions and immunodeficiency in various instability syndromes such as Fanconi Anemia, Bloom Syndrome, Ataxia Telangiectasia and Roberts syndrome all of which have increased risks of developing malignancies (Zhang 2005).

2.2.Measurement of chromosomal instability

CIN needs to be characterized to measure the chromosomal variations between cells across a given population and also to assess the rate at which these variations change. Fluorescence *in situ* hybridization (FISH) has been used to assess CIN. An accepted method for indirectly inferring the nCIN using chromosome enumeration probes in FISH

analysis rate has been developed (Speicher and Carter 2005, Geigl et al. 2008, McGranahan et al. 2012). In this method, the nCIN rate is inferred by determining the percentage of cells exhibiting a chromosome signal number that deviates from the modal chromosome number. G-banding, FISH with regional/chromosome specific probes, spectral karyotype (SKY) analysis and Comparative Genomic Hybridization are cytogenetic methods for detecting sCIN. More recently, bioinformatics studies to detect changes in chromosome structure and number have been developed (McGranahan et al. 2012, Carter et al. 2006). With the advent of next generation sequencing technology, it will be interesting to see how these tools will be exploited in determining CIN at the level of nucleotide sequence (McGranahan et al. 2012).

2.3.Chromosomal instability in cancer: a benefit and a vulnerability

CIN is a characteristic feature of most solid tumors and hematologic human cancers (McGranahan et al. 2012). It was first demonstrated in colorectal cancer cell lines by Vogelstein and his colleagues in 1997 (Lengauer, Kinzler, and Vogelstein 1997). CIN is associated with unfavorable prognosis in human cancer resulting in poor patient outcomes (McGranahan et al. 2012, Carter et al. 2006). CIN levels in tumors can stratify patients where high levels of CIN are generally linked to poorer survival and increased risk of relapse (McGranahan et al. 2012, Carter et al. 2006, Slee et al. 2014).

CIN is proposed to be involved in the initiation and growth of tumors causing aneuploidy and intra-tumor heterogeneity (Bakhoum and Compton 2012, McGranahan et al. 2012).

Cancer cells that have elevated nCIN are reported to have elevated chromosomal mis-segregation levels altering gene dosage and promoting Loss of Heterozygosity (LOH) events (Thompson and Compton 2008, Lengauer, Kinzler, and Vogelstein 1997, Bakhoum and Compton 2012). These genetic changes provide an advantage to cancer cells and allow them to tolerate and adapt to the stress present in the environment and facilitate emergence of drug resistant cells (McGranahan et al. 2012). However, this instability itself imposes stress and makes cancer cells potentially vulnerable (Gordon, Resio, and Pellman 2012). It may even play a role in tumor suppression if CIN gets too high by putting stress on the cells (Gordon, Resio, and Pellman 2012).

CIN is reported to be a 'double-edged sword' where in addition to conferring tumor cells with selective advantages, it negatively impacts their biological fitness (Williams et al. 2008). A threshold exists in the tolerance of CIN by cancer cells beyond which it is no longer compatible with cellular viability (Cahill et al. 1999). Elevated levels of chromosome segregation errors have been linked with DNA damage in the form of double strand breaks (DSBs) which lead to unbalanced translocations in daughter cells (Janssen et al. 2011). Daughter cells undergo cell death when the DSBs are not efficiently repaired or the gene imbalance as a result of the translocation is too severe for the cell (Janssen and Medema 2011). CIN results in the accumulation of deleterious mutations arising as a result of genomic rearrangement (Kops, Foltz, and Cleveland 2004, Janssen, Kops, and Medema 2009, Cahill et al. 1999). Earlier studies in yeast and mice have shown that the addition of an extra chromosome adds to the burden on the energy requirements of the cells because the genes on the extra chromosomes are being

replicated and transcribed slowing down cell growth and increasing cell lethality (Torres et al. 2007, Williams et al. 2008). It has been observed that cells make attempts to correct altered protein stoichiometry when chromosomally unstable (Tang et al. 2011). Pathways that stabilize normal cells under environmental stress are defective in tumor cells making them more vulnerable to stress inducing agents (Cahill et al. 1999). In a recent clinical study, extreme genomic instability in breast cancer was associated with favorable prognosis which might be explained by the vulnerable nature of cancer cells with an extremely high CIN level (Birkbak et al. 2011). Of interest to the hypothesis of this thesis, we test whether it is possible to selectively target CIN often seen in cancer cells to promote CIN elevation in them resulting in their death, while sparing normal cells.

3. Mitotic kinase protein MPS1 as a potential therapeutic target

3.1.Spindle Assembly Checkpoint Complex:

Mitosis is a highly regulated process governed by the spindle assembly checkpoint (SAC) (also called Mitotic Checkpoint Complex (MCC)) to ensure legitimate segregation of chromosomes to the daughter cells. The role of the SAC, which is a large protein complex that binds to kinetochores, is to ensure proper alignment of the chromosomes at the metaphase plate and regulate bipolar attachment of the sister chromosomes to the mitotic spindle thus giving the green signal for the transition of the cells from metaphase to anaphase (Musacchio and Salmon 2007).

Unattached and/or misaligned chromosomes signal the SAC to block the anaphase promoting complex (APC/C) by inactivating its activator cdc20 (Yu 2007, 2002). APC/C is an ubiquitin ligase that promotes the progression of mitosis from metaphase to anaphase. When the SAC is activated, it prevents the polyubiquitylation and proteasome mediated degradation of cyclin B and securin, both of which are bound to CDK1 and separase respectively arresting mitosis in prometaphase. On degradation of cyclin B, CDK1 is inactivated thereby promoting progression of mitosis whereas when securin is degraded, it releases separase which in turn cleaves the SCC1 component of the cohesin complex that binds the sister chromatids together. This event allows the sister chromatids to separate to opposite poles allowing anaphase to progress (Peters 2006). Figure 5 shows the key components and the functional mechanism of the SAC in the transition of prometaphase to anaphase [Figure 5 adapted from (Colombo and Moll 2010)].

3.2.Function of MPS1 kinase

The SAC is a complex of proteins and one important component of this complex is monopolar spindle 1 (MPS1) kinase, also known as TTK (Colombo et al. 2010). MPS1 is a cell cycle regulated dual serine/threonine kinase and also phosphorylates tyrosine residues (Stucke et al. 2002). It is a highly dynamic kinase and is expressed only in proliferating cells with maximum activity during mitosis (Stucke et al. 2002, Colombo et al. 2010). The activity of MPS1 kinase increases with the activity of the SAC (Stucke et al. 2002). Though its exact functions remain unknown, MPS1 kinase is reported to be involved in the maintenance of the SAC as well as in stabilizing the attachment of the

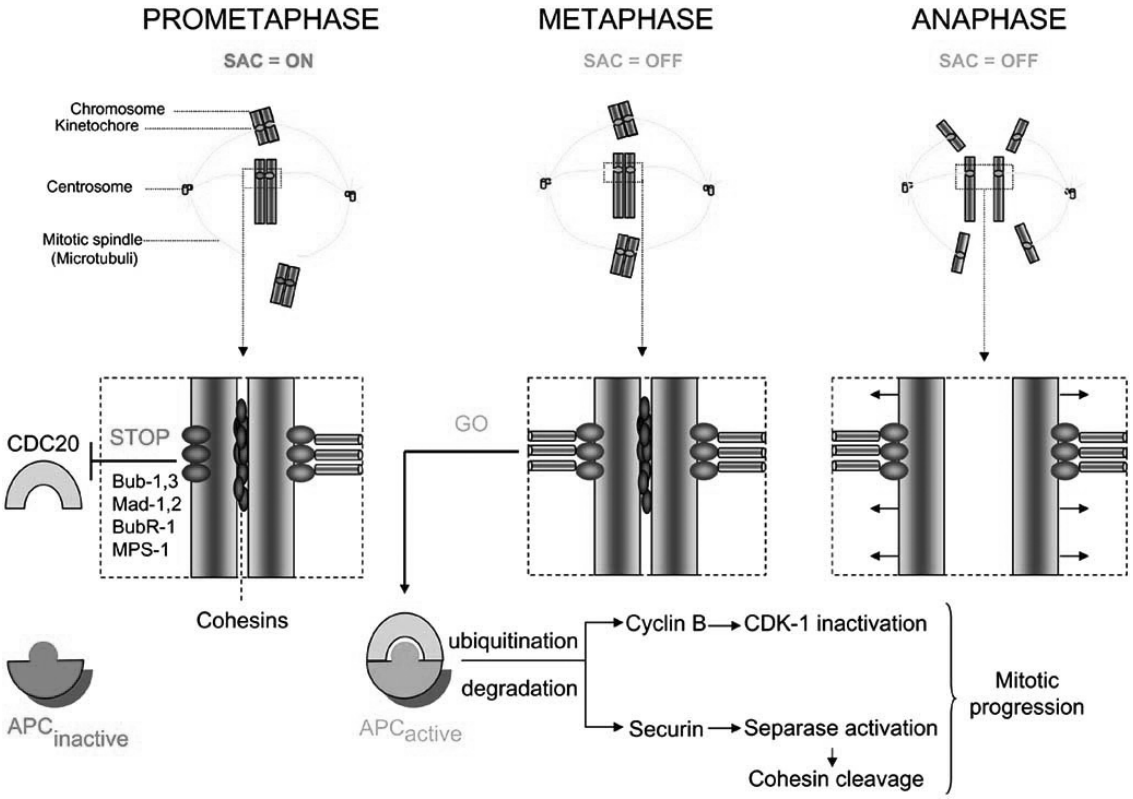

Figure 5 Key components and their function in Spindle Assembly Checkpoint

Before metaphase, unattached chromatids trigger the Spindle Assembly checkpoint signal which comprises of BUB1, BUB3, MAD1, MAD2, MPS1 and BUBR1. This signal inhibits the APC/C by blocking cdc20. Once all the kinetochores are attached to the spindle microtubules, the SAC is released from kinetochores resulting in activation of APC/C. This in turn, degrades cyclin B and securin resulting the separation of sister chromatids and progression of the cell cycle into anaphase.

[Figure adapted from (Colombo and Moll 2010)]

mitotic spindle fibers to the kinetochores (Janssen, Kops, and Medema 2009, Weiss and Winey 1996, Stucke et al. 2002). In addition, it localizes to the kinetochore and is required to recruit essential SAC proteins such as MAD1 and MAD2 to the kinetochore (Stucke et al. 2002, Liu et al. 2006, Tighe, Staples, and Taylor 2008, Kang et al. 2007). MPS1 inhibits post-mitotic checkpoint activation of the p53 signaling pathway in the event of controlled and normal chromosomal segregation (Tardif et al. 2011). In chromosomally unstable cancer cells, MPS1 kinase inhibition causes massive aneuploidy, which is incompatible with survival making it an excellent target in selective cancer therapeutics (Tighe, Staples, and Taylor 2008, Jelluma, Brenkman, van den Broek, et al. 2008). Interestingly, normal fibroblasts and other differentiated cells are less affected thus creating a possible therapeutic window for treatment against a wide range of cancers (Colombo et al. 2010, Slee et al. 2014).

3.3.Elevated Expression of MPS1 in chromosomally unstable cancer cells

CIN is tolerated by cancer cells until a threshold is reached, beyond which it proves to be lethal to the cell. To maintain CIN within the survivable limits as well to reap the benefits provided by CIN, it is proposed that cancer cells up-regulate genes that are critical to limit chromosomal segregation errors (Grabsch et al. 2003, Schmidt and Medema 2006, Yuan et al. 2006). Carter and his group identified a set of 70 genes, called the CIN70 signature, which in an unbiased bioinformatics approach turned out to stratify patients into groups based on survival or risk of relapse (Carter et al. 2006). In general, patients with high levels of CIN70 (which serves also as a surrogate measure of CIN) have a

poorer prognosis than those with lower CIN70 gene expression levels (Carter et al. 2006, Birkbak et al. 2011). CIN70 genes play a role in chromosome maintenance and include 3 SAC genes. It is hypothesized that CIN70 gene up-regulation keeps CIN within limits to allow cancer cell survival. This increased reliance of tumor cells to elevate genes involved in chromosome maintenance is a form of "non-oncogene addiction" (Luo, Solimini, and Elledge 2009, Solimini, Luo, and Elledge 2007). Almost a quarter of these 70 genes are associated with chromosomal segregation including MPS1 kinase (Carter et al. 2006, Luo, Solimini, and Elledge 2009).

3.4.Inhibition of MPS1 as a therapeutic target in cancer cells

MPS1 kinase activity is required in dividing cells for maintenance of the SAC and to ensure correct segregation of chromosomes. It is reported that inhibition of MPS1 kinase results in the override of the SAC and premature exit from mitosis (Schmidt et al. 2005, Kwiatkowski et al. 2010, Burgess, Rasouli, and Rogers 2014). Silencing of this mitotic kinase in mammalian cells has been shown to generate massive chromosomal mis-segregation due to inaccurate alignment of chromosomes at the metaphase plate and accelerated mitosis ultimately leading to cell death (Tighe, Staples, and Taylor 2008, Jelluma, Brenkman, van den Broek, et al. 2008). Yeast cells that harbor mutations resulting in faulty chromosomal segregation are more sensitive to compounds that inhibit MPS1 kinase (Dorer et al. 2005). Many groups have demonstrated in a wide variety of tumor cell lines including cancer cells derived from lung, ovary and colon that targeted inhibition of MPS1 makes these cancer cells die via massive chromosome mis-

segregation (Jemaa et al. 2012, Kwiatkowski et al. 2010, Castedo et al. 2004). The inhibition impairs SAC and kills human cancer cells by inducing hyperdiploidization, arresting growth and inhibiting DNA synthesis causing gross aneuploidy followed by cell death making MPS1 a potential therapeutic approach in cancer as normal cells are less affected (Jemaa et al. 2012, Tardif et al. 2011, Kwiatkowski et al. 2010, Slee et al. 2014, Colombo et al. 2010).

One such MPS1 inhibitor [Figure 6 adapted from (Colombo et al. 2010)], NMS-P715, an ATP competitor was identified and characterized as a small-molecular inhibitor, which is selective towards cancer cell lines. NMS-P715 accelerates mitosis and affects localization of kinetochore components leading to massive aneuploidy and cell death leaving normal cells almost unaffected [Figure 7 adapted from (Colombo et al. 2010)]. The molecule is also shown to rapidly inhibit tumor growth in mouse xenograft models for ovarian carcinoma and malignant melanoma [Figure 8 adapted from (Colombo et al. 2010)].

4. Targeting pancreatic cancer cells

Pancreatic ductal adenocarcinoma (PDAC) is a deadly cancer of the exocrine pancreas, a functional unit of the pancreas that produces digestive zymogens and forms 80% of the total tissue mass of the organ (Hezel et al. 2006). It is the most common form of pancreatic cancer and has an abysmal five year survival rate of less than 6% (Siegel, Naishadham, and Jemal 2012). It is the 4th highest contributor to cancer related deaths in the United States and there has been little improvement in patient survival over the last

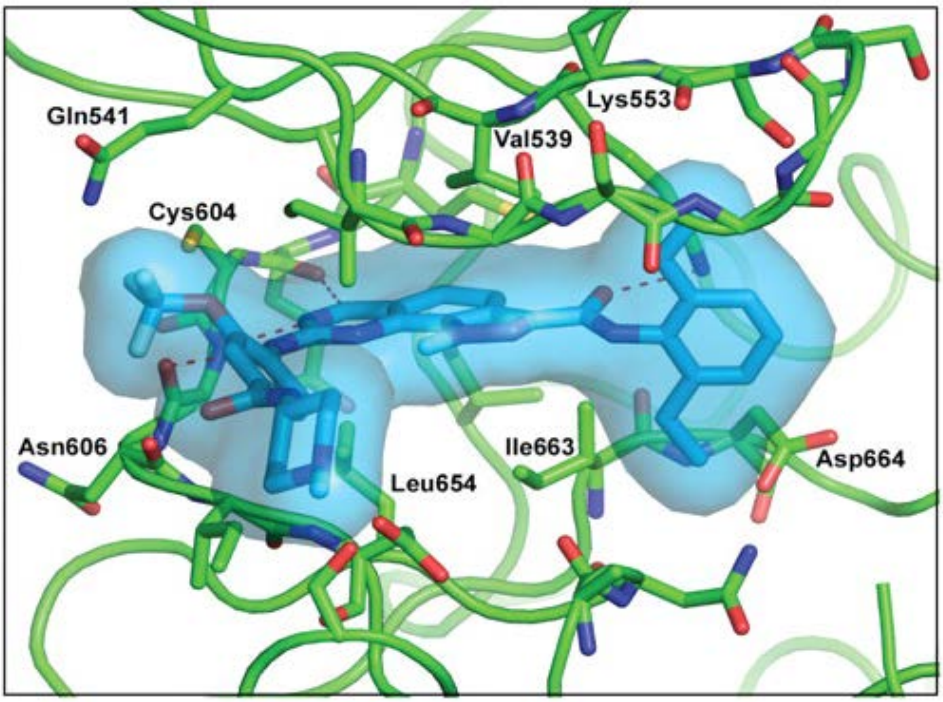

Figure 6 NMS-P715 is a selective ATP-competitor that inhibits MPS1

NMS-P715 (shown in light blue competitively binds in the catalytic domain of MPS1 kinase (shown as sticks with green carbon). Hydrogen bonds between NMS-P715 and MPS1 are represented by red dashed lines.

[Figure adapted from (Colombo et al. 2010)]

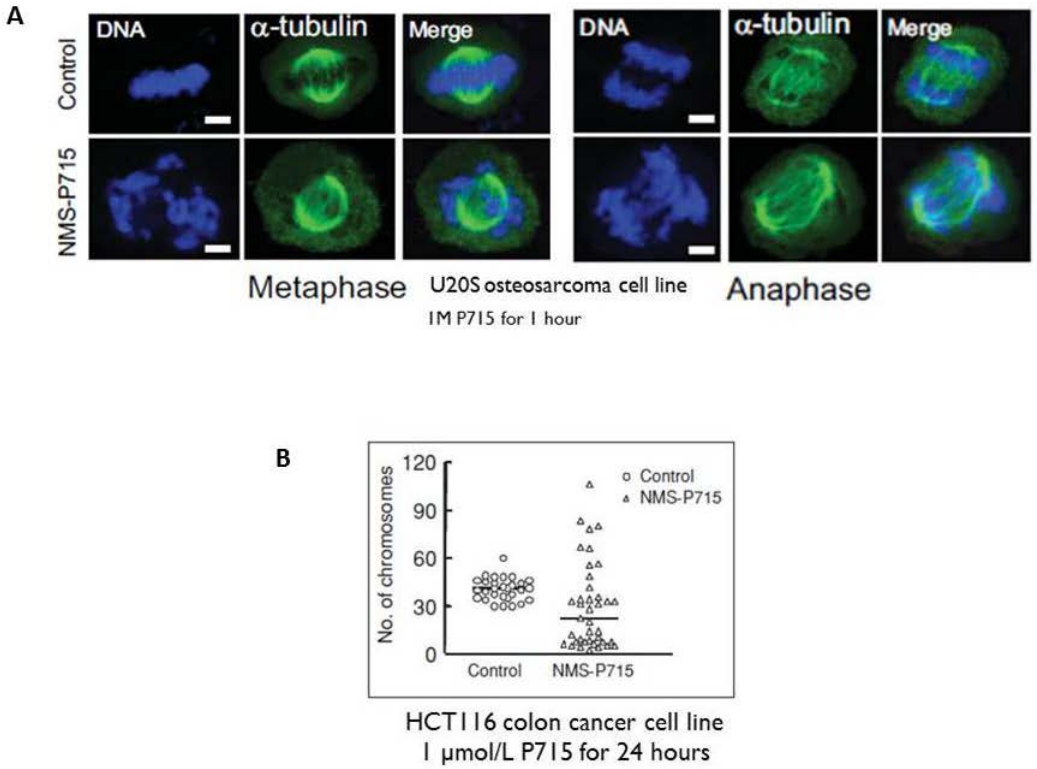

Figure 7 NMS-P715 inhibits Spindle Assembly Checkpoint affecting alignment of chromosomes and subsequently increases aneuploidy in treated cells

A: Metaphase and anaphase images of U2OS osteosarcoma cell line that are treated with DMSO (control) or 1 µmol/L NMS- P715 for 1 hour. DNA is stained with DAPI (blue) while alpha-tubulin is visualized as green fluorescent signal YFP.

B: Mitotic chromosome spread results of HCT116 colon cancer cells treated with NMS-P715 (1 µmol/L) or DMSO (control) for 24 hours. Treated cells show a wide range of chromosome numbers/ cell consistent with increased aneuploidy after treatment with the inhibitor.

[Figure adapted from (Colombo et al. 2010)]

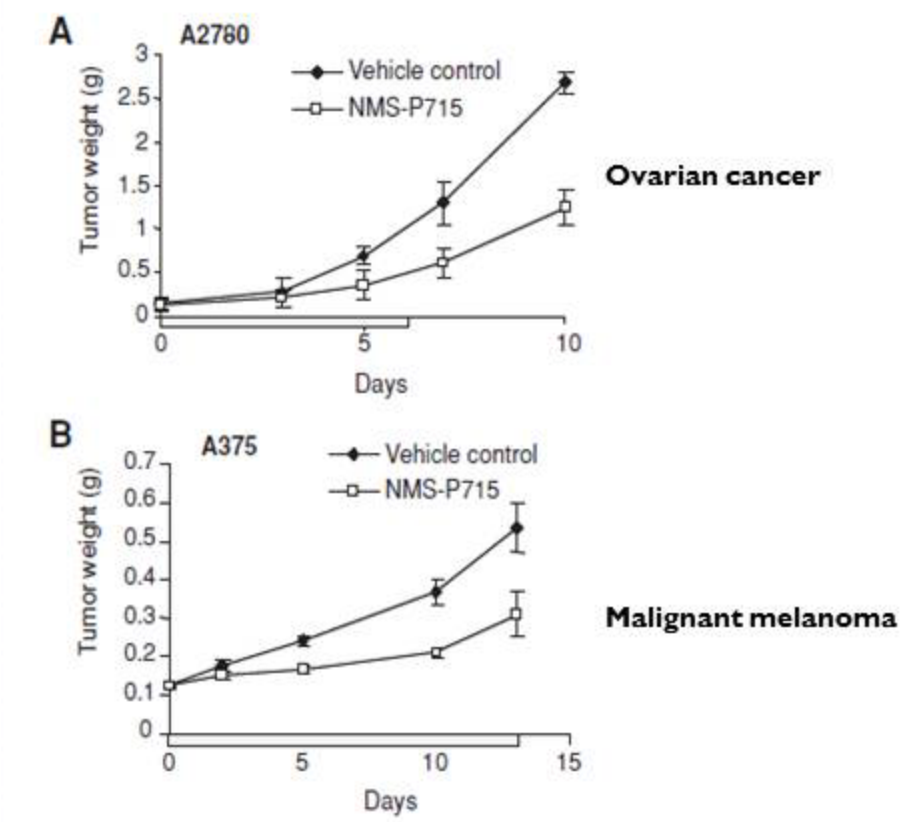

Figure 8 NMS-P715 inhibits growth of tumors in xenograft models

A: Nude mice bearing xenograft derived ovarian cancer tumors (A2780human cell line) show a significant reduction of tumor weight after oral administration of 90mg/kg NMS-P715 (gray bar) daily for 7 consecutive days when compared to the control group.

B: A375 human melanoma xenograft model also showed significant decrease in tumor weight after they were orally administered with 100mg/kg NMS-P715 for 2 consecutive days over a 10 day period as compared to the control group.

[Figure adapted from (Colombo et al. 2010)]

30 years (Siegel, Naishadham, and Jemal 2012). Pancreatic cancer is typically detected at a later stage as the disease is symptom-free in the early stages and has a high metastatic propensity making surgery and other therapeutic interventions inadequate (Karhu, Mahlamaki, and Kallioniemi 2006). The current compound used in PDAC treatment, the DNA synthesis inhibitor gemcitabine, does little to improve the overall survival (Choi, Saif, and Kim 2014). This stresses the need to develop new therapeutic options that improve survival of patients suffering from PDAC.

PDAC tumor cells are genetically very complex, show extensive heterogeneity, and are highly unstable leading to acquired clonal aberrations and complex karyotypes (Karhu, Mahlamaki, and Kallioniemi 2006, Gorunova et al. 1998). The most common mutation that is observed in over 90% of PDAC tumors is the activating mutation of KRAS oncogene (Hansel, Kern, and Hruban 2003). Elevated CIN70 gene expression, including that of MPS1 kinase, in PDAC patient tumor samples indicated poor prognosis (Slee et al. 2014). Patients with PDAC were categorized into prognostic categories based on their MPS1 kinase expression levels [Figure 9 adapted from (Slee et al. 2014)]. In addition, MPS1 kinase was highly expressed in PDAC cell lines such as BxPC3 and PANC-1 in comparison with primary pancreatic ductal epithelial cells [Figure 10 adapted from (Slee et al. 2014)]. Due to the presence of CIN and elevated MPS1 kinase levels, we explored the potential of NMS-P715 to 1) inhibit PDAC cell growth and 2) have less effect on stem cells, as these cells are the main target of cytotoxic compounds widely used in chemotherapy.

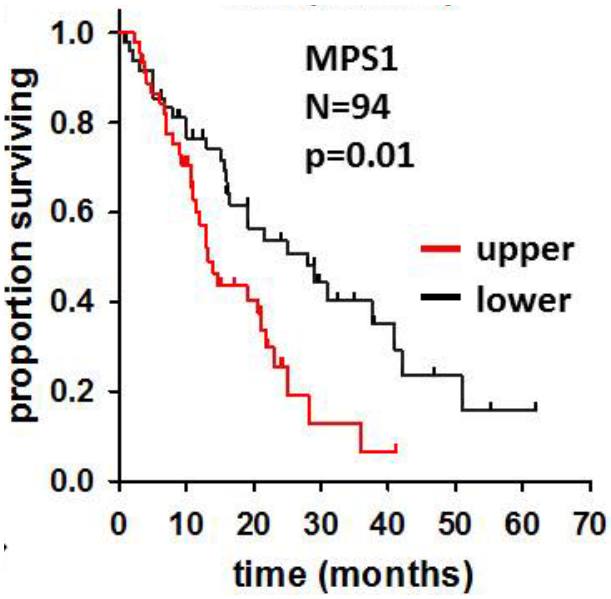

Figure 9 Kaplan-Meier Survival analyses of 94 PDAC patients segregated by level of MPS1 expression

The graph represents the survival analysis of 94 PDAC patients relative to levels of MPS1 expression. The patients were classified in two prognostic categories namely the 'upper' and 'lower' halves based on the expression of MPS1. The median survival for upper half patients is 28 months whereas that for the lower half is 13.2 months.

[Figure adapted from (Slee et al. 2014)]

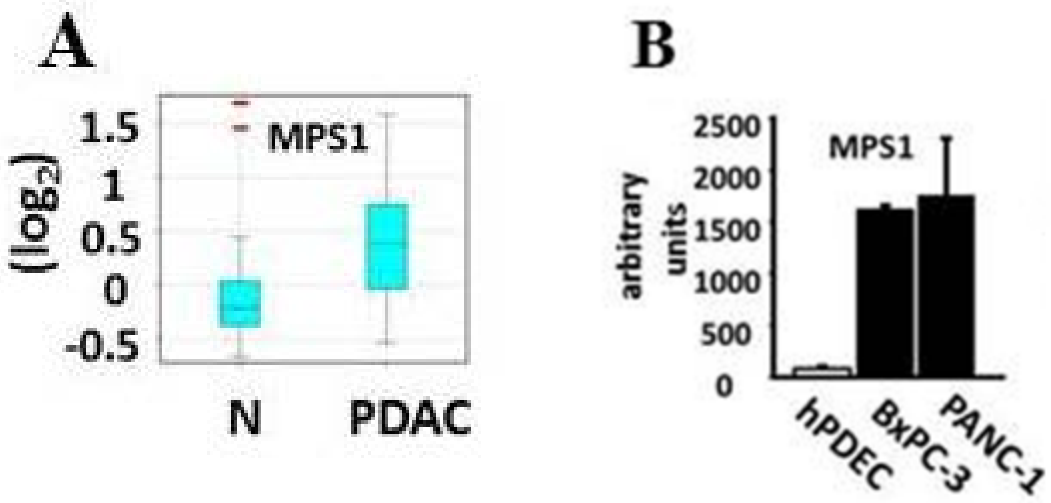

Figure 10 MPS1 is up-regulated in pancreatic cancer

A: Box whisker plot of MPS1 expression in normal pancreatic cells and PDAC cells. Central band indicates the median and the lower and upper bands denote first and the third quartiles respectively. Whiskers represent the data within the 1.5 interquartiles of upper and lower box limits. Red bars indicate outliers.

B: MPS1 gene expression in primary human pancreatic ductal epithelial cells (hPDEC), BxPC-3, and PANC-1 cell lines**.**

[Figure adapted from (Slee et al. 2014)]

5. Human Adipose Stem Cells

Therapeutic Index is defined as the ratio of the concentration of the drug required for the toxic effects to the concentration required for therapeutic effects (Kaelin 2005). A drug is reported to have a favorable therapeutic index when it targets a biomolecule that is important for the survival of a cancer cell without compromising normal cellular survival or function (Rubin and Gilliland 2012). Most of the chemotherapeutic compounds in use today, including gemcitabine, have very low therapeutic indices as they damage normal cells such as the stem cells of the bone marrow and the normal epithelia of the gut (Kaelin 2005). As a result, the success rate of most anticancer drugs remains low (Rubin and Gilliland 2012). NMS-P715 is shown to have a favorable therapeutic index in untransformed cells such as fibroblasts and B-lymphocytes as they are almost unaffected by it under conditions where cancer cells are severely affected (Colombo et al. 2010). Since NMS-P715 was not tested in stem cells, we wanted to check whether it inhibited MPS1 kinase in adipose derived mesenchymal stem cells (ASCs) as a model for how stem cells might be affected *in vivo*.

ASCs are multipotent stem cells that were isolated from donors who underwent liposuction procedures. They share many properties with the bone marrow derived mesenchymal stem cells (Hong, Traktuev, and March 2010). These cells have high proliferation capacity *in vitro* and maintain a stable diploid karyotype (Grimes et al. 2009). As gemcitabine affects stem cells *in vivo*, the relative sensitivities of pancreatic cancer cells and ASCs to both Gemcitabine and NMS-P715 were analyzed by Dr. Roger

B. Slee from Grimes' Lab. Pancreatic cancer cell lines such as PANC-1 and BxPC-3 were significantly more sensitive to the inhibitory effects of NMS-P715 than five isolates of ASCs when treated with increasing concentrations of the drug for 72 hours [Figures 11 A and 11B adapted from (Slee et al. 2014)]. This was also reflected in the IC_{50} values which were 1.5, 1.6 and 3.4 µmol/L for PANC-1, BxPC-3 and ASCs respectively [Figure 11C adapted from (Slee et al. 2014)]. These results when compared to those observed in the PDAC cells were more resistant than ASCs suggesting a favorable therapeutic index for the MPS1 kinase inhibitor (Slee et al. 2014). Based on the results established in the previous studies and Dr. Slee's experiments, we hypothesize that MPS1 inhibition is selective towards PDAC cells and that stem cells will be more resistant *in vitro*. In addition, we also tested whether normal human pancreatic ductal epithelial (hTERT-HPNE) cells immortalized by human telomerase, were negatively affected by the inhibition of MPS1 kinase. The inhibitor was tested in murine PDAC cells (Carriere et al. 2011). The results presented in this thesis focus on the relative resistance of stem cells and normal pancreatic epithelial cells to the NMS-P715 inhibitor of MPS1 kinase compared to pancreatic cancer cells and support further testing of MPS1 inhibitors as potential selective agents for treatment of pancreatic cancer.

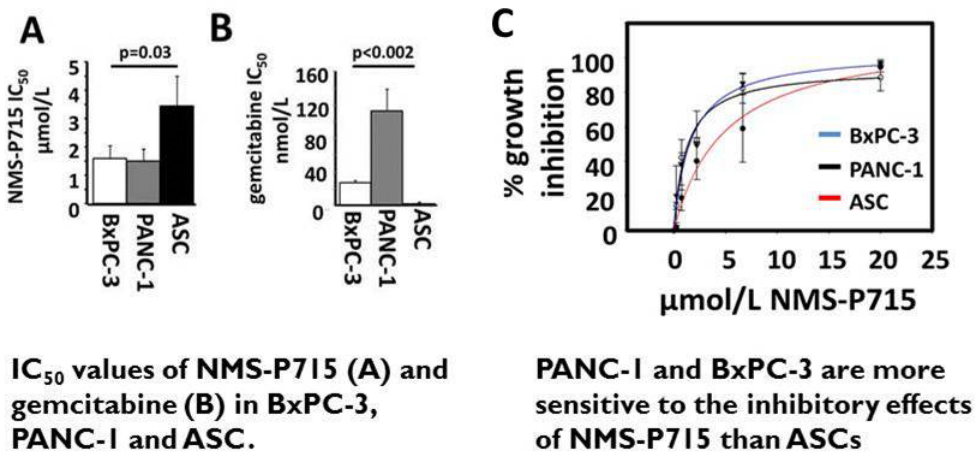

Figure 11 Sensitivities of Pancreatic Cancer Cells and ASCs to both gemcitabine and NMS-P715

A: IC50 values of NMS-P715 in BxPC-3, PANC-1 and ASC

B: IC50 valued of gemcitabine in BxPC-3, PANC-1 and ASC

C: PANC-1 and BxPC-3 are more sensitive to the inhibitory effects of NMS-P715 than ASCs

[Figure adapted from (Slee et al. 2014)]

MATERIALS AND METHODS

1. Cell lines

Human PDAC cell lines PANC-1 (ATCC CRL-1469) and BxPC-3 (ATCC CRL-1687) were grown in Dulbecco's Modified Eagle Medium/10% FBS or RPMI/10% FBS, respectively, obtained from American Type Culture Collection (ATCC). 825-2 and 1170-4 KRC cells are murine PDAC cells that were obtained from two pancreatic cancer tumors arising in a genetically engineered mouse model of PDAC in which *Kras* was combined with *Rb* (Retinoblastoma) gene deletion using *Cre*-recombinase (Carriere et al. 2011) and were cultured in RPMI/10% FBS. Human ASCs were collected from donors undergoing lipoaspiration using an approved protocol (Institutional Review Board 0305-59) as described previously (Hong, Traktuev, and March 2010) and cultured in EGM2-MV medium (Lonza)/ 10% FBS. Human telomerase-immortalized pancreatic ductal epithelial cells (hTERT-HPNE) cells (ATCC CRL-4023) were cultured according to the supplier's conditions. The cells were maintained in a humidified atmosphere of 5%CO_2 at 37°C.

The population doubling times of PANC-1, BxPC-3, KRC, ASC and hTERT-HPNE cells were approximately 50 hours, 40 to 60 hours, 20 hours, 24 to 26 hours and 40 hours respectively.

2. Compounds

NMS-P715 was provided by Nerviano Medical Sciences or purchased from EMD Millipore (Cat#475949-5MG) and suspended in dimethyl sulphoxide (DMSO). Gemcitabine, obtained from Tocris Bioscience, was suspended in H_2O.

3. Clonogenic Survival Assay

Cells were counted and plated in duplicate or triplicate in 12 well dishes. The inhibitor was added after 24 hours at concentrations ranging from 0.1 to 5.0 µmol/L. DMSO was used as a control. For continuous treatment, the inhibitor was added every 3 days whereas in washout condition the cells were treated with the inhibitor for 24 hours and then grown in compound-free medium. The total duration of cell growth was 9 days or 6 days after the first addition of the drug. Cells were fixed with methanol for 15 minutes and left to dry overnight. The plates were then stained with 0.05% methylene blue (w/v) for 15 minutes, rinsed twice in water and dried overnight. Cell growth was quantified by suspending cells in 0.5 mol/L hydrochloric acid and the optical density was measured at 320 nM on a Beckman-Coulter DTX880 MultiMode Detector (Oliver et al. 1989). Inhibition of growth by NMS-P715 was measured relative to the DMSO control.

4. SAC Override analysis by Immunoblotting Assay

4.1.Preparation of Cell Lysate

Cells were treated with increasing concentrations of NMS-P715 or DMSO control for 72 hours after which, media was aspirated from cultures. Then cells were harvested by adding 1ml 0.25% Trypsin (Thermo Scientific) and collected in graduated 15 ml centrifuge tubes (Sarstedt). The cells were washed with 1X PBS (Dulbecco), counted using Beckman Coulter Counter and frozen at -80°C overnight in medium supplemented with 10% DMSO until next use. After thawing the cells on ice, the cells were lysed by resuspension in RIPA buffer (Thermo Scientific) and sonication for 30 seconds at 10 second intervals to shear DNA and reduce viscosity. A 15µl aliquot removed then 15µl RIPA buffer, 15µl 3X Loading Buffer and 1µl 2-Mercaptoethanol (BME) were added then the sample was heated for 3 minutes at 90°C followed by cooling on ice.

4.2.SDS-polyacrylamide gel electrophoresis of protein and transfer:

30µl of sample was loaded onto SDS-PAGE gel (10cm X 8.5cm) (Thermo Scientific) and electrophoresis was performed. After separation on the gel, proteins were electroblotted onto Polyvinylidene fluoride (PVDF) membrane (Biorad) overnight at 30V at 4°C. The transfer buffer contained 30mM Tris, 200mM glycine, 1.4mM SDS and 20% methanol.

4.3.Immunoblotting

The membranes were incubated while under constant agitation in blocking buffer [3% (w/v) non-fat dry milk in PBS] at room temperature for 1 hour. After washing briefly in 1XPBS-T buffer (1X PBS / 0.05% Tween), membranes were immunoblotted in blocking buffer containing primary antibody [phosphorylated histone H3 serine 10 (pS10H3); (Millipore)] at a concentration of 1:1000 (v/v) at room temperature for 2 hours. Membranes were washed 3 times, each wash for 10 minutes, with 1XPBS-T buffer, followed by incubation in blocking buffer containing goat-anti-rabbit-horseradish-peroxidase conjugates [1:5000 (v/v)] for 1 hour at room temperature. After three washes (10 minutes each wash) in 1XPBS-T buffer, the membranes were exposed to enhanced chemilumescent (ECL) plus mixture (Thermo Scientific Pierce) for 1 minute, and exposed to autoradiographic film from 10 seconds to 5 minutes to obtain desired signal intensity. The same procedure was followed for a loading control using β-Actin (Sigma) [1:10000 (v/v)] as the primary antibody and goat-anti-mouse-horse radish peroxidase conjugate [1:5000 (v/v)] as the detecting antibody.

5. SAC Override Assay by Immunofluorescence

5.1.Cell Fixation

PANC-1 and BxPC-3 Cells were counted and plated in chamber slides at a concentration of 10,000 to 20,000 cells/well. After 24 hours, replicate cultures were blocked in

75nmol/M nocodazole for 18 hours. The cells were treated either with DMSO or 0.4 µmol/L NMS-P715 in the last two hours of block. Murine PDAC cells were plated at same densities as that of human PDAC cells but were treated with increasing concentration of nocodazole for 18 hours without NMS-P715 treatment. DMSO was used as control. The cells were then fixed in 1XPBS/4% formaldehyde for 15 minutes at room temperature and washed three times in 1XPBS for 5 minutes each.

5.2.Immunofluorescence

The cells were blocked for 60 minutes in 100 µl of blocking buffer containing 1X PBS, 5% normal goat serum and 0.3% Triton X-100. The cells were then incubated overnight at 4°C with Alexa-Fluor 488-labeled pS10H3 antibody (Cell Signaling Technologies) which was diluted in the antibody dilution buffer (1x PBS/ 1% bovine serum albumin (BSA)/ 0.3% Triton X-100) in a dark moist chamber. After incubation, the cells were rinsed thoroughly with PBS three times for 5 minutes each at room temperature. The nuclei were counterstained with 6-diamidino-2-phenylindole (DAPI) and observed under a Leica DM5000B fluorescence microscope (Leica Microsystems). A minimum of 200 cells per well were scored for the presence of green pS10H3 positive cells. The assay was performed in duplicates.

6. Measuring nCIN using Fluorescence *In Situ* Hybridization

6.1.Cell Fixation

Duplicate cell cultures were incubated with NMS-P715 or DMSO control for 72 hours after which they were harvested using 1 ml 0.25% Trypsin/EDTA) and centrifuged at 1000 rpm for 5 minutes. The cells were incubated for 10 minutes at 37°C in hypotonic buffer (0.075M KCl). The cells were then fixed in 3:1 (v/v) methanol: acetic acid, dropped on slides and aged under vacuum overnight.

6.2.Preparation of FISH probes

Commercial probes recognizing X chromosome or chromosome 17 centromeres (Abbott Molecular Cat# 05-J08-033, 06-J37-027 respectively) in human cells were aliquoted into hybrisol containing 50%formamide, 20%dextran sulfate, 2XSSC and 0.1mg/ml salmon sperm DNA. The probes were denatured at 72°C for 7 minutes, and then placed on ice in dark.

For murine cells, commercial probe for mouse chromosome 11qE1 (Kreatech Diagnostics Cat#30501) was aliquoted in mouse probe solution containing ultrapure deionized water and large-scale integration (LSI) hybridization buffer provided by the supplier. The probe was denatured at 73°C for 5 minutes and placed on ice in dark prior to adding to slides.

6.3.Hybridization

The slides containing the cells were preheated in 2X SSC at 37°C for approximately 30 minutes. The slides were washed three times in 70%, 80% and 100% ethanol sequentially for 2 minutes each at -20°C and dried at room temperature. The slides were placed in denature solution (70%Formamide/2XSSC; pH 7.0) for 2 minutes at 72°C to denature DNA. The cells were again sequentially washed with ethanol at -20°C and dried at room temperature. 15µl of the human probe mixture or 10µl of the mouse probe was added to the samples and sealed in a coverslip. The slides were incubated overnight in a moist chamber at 37°C.

After incubation, the PDAC and ASC slides were placed twice in post hybridization wash solution (50%Formamide/2XSSC; pH 7.0) at 42°C for 8 minutes each followed by a wash in 2XSSC at 37°C for 8 minutes. KRC slides were placed in post hybridization wash I (0.4X SSC/0.3%NP40) at 73°C for 2 minutes followed by wash in post hybridization wash II solution (2X SSC/ 0.1%NP40) at room temperature for 1 minute.

The nuclei were counterstained with 6-diamidino-2-phenylindole (DAPI) and observed under Leica DM5000B fluorescence microscope (Leica Microsystems). Chromosome numbers were counted in ≥50 spreads per culture.

RESULTS

1. Pancreatic cancer cell growth is selectively inhibited by MPS1 inhibitor NMS-P715 whereas human stem cells are markedly less affected.

To examine the specificity of MPS1 inhibition by NMS-P715, we tested and compared the effects of the drug in pancreatic cancer cells with that in the normal adipose stem cells (ASCs) using a clonogenic survival assay. The assay assesses the ability of the cells to divide and proliferate after an external insult to them (Puck and Marcus 1956). This long term assay is an indirect measurement of cell death *in vitro* where it takes into account the different mechanisms that kill cancer cells (Brown and Attardi 2005). This is observed as a decrease in the cell number after the treatment under consideration. To analyze the growth inhibitory effects of the drug, we checked for the proliferative capacity of the cells after treatment with NMS-P715. The assay requires the formation of large, deeply staining colonies each consisting of more than 50 cells as an indicator that the cells have retained their clonogenic properties (Puck and Marcus 1956). Hence, the optimum seeding number and length of treatment is different for each cell line and experiment to obtain the best representative results in the clonogenic survival assay.

PANC-1 and BxPC-3 cells were seeded at concentrations of 500 and 1000 cells/well respectively. When PDAC cells were treated continuously for 9 days with NMS-P715 at concentrations ranging from 0.1 µmol/L to 5.0 µmol/L, their growth was markedly inhibited at 0.5 µmol/L (Figure 12A). In addition, a treatment of 1.0 µmol/L NMS-P715

for 24 hours followed by compound washout significantly reduced the proliferation of PANC-1 and BxPC-3 indicating high sensitivity to the drug (Figure 12A). On the other hand, ASCs were more resistant when they were plated at a density of 200 cells/well and treated with the drug for 6 days continuously at increasing concentrations from 0.1 µmol/L to 5 µmol/L (Figure 12B). The stem cells show a marginal decrease in cell growth at 0.5 µmol/L when compared to that in 0.1 µmol/L but are still actively proliferating which is in contrast to that observed in PDAC cells.

However since ASCs do not form colonies at low plating numbers because they are migratory (Figure 12B), cell growth was measured using an indirect colorimetric assay to quantitatively compare the growth of PDAC cells with ASCs after MPS1 inhibition. In this assay, cells were fixed and stained with methylene blue. The dye was released by lowering the pH with hydrochloric acid and the intensity of the color of the extracted dye was measured as the absorbance on a plate reader (Oliver et al. 1989). PANC-1, BxPC-3 and ASC were plated at a density of 200cells/well and treated with NMS-P715 at concentrations varying from 0.1 µmol/L to 0.5 µmol/L for 6 days. A clear trend is visible where the number of cells surviving after treatment decreases with the increasing concentration of the drug (Figure 12 C). The percentage of PDAC cells surviving decreases sharply in comparison with that of the ASCs. Also, the PDAC cells seem to be more sensitive than the ASCs to MPS1 inhibition and there is significant difference between their survivals when treated with 0.3 µmol/L, 0.4 µmol/L and 0.5 µmol/L NMS-P715. In addition, the growth inhibition of PANC-1 (75.1% ± 7.7%) and BxPC-3 (78.83% ± 4.49%) was twice that observed in the ASCs (38.2% ± 8.5%). These data are

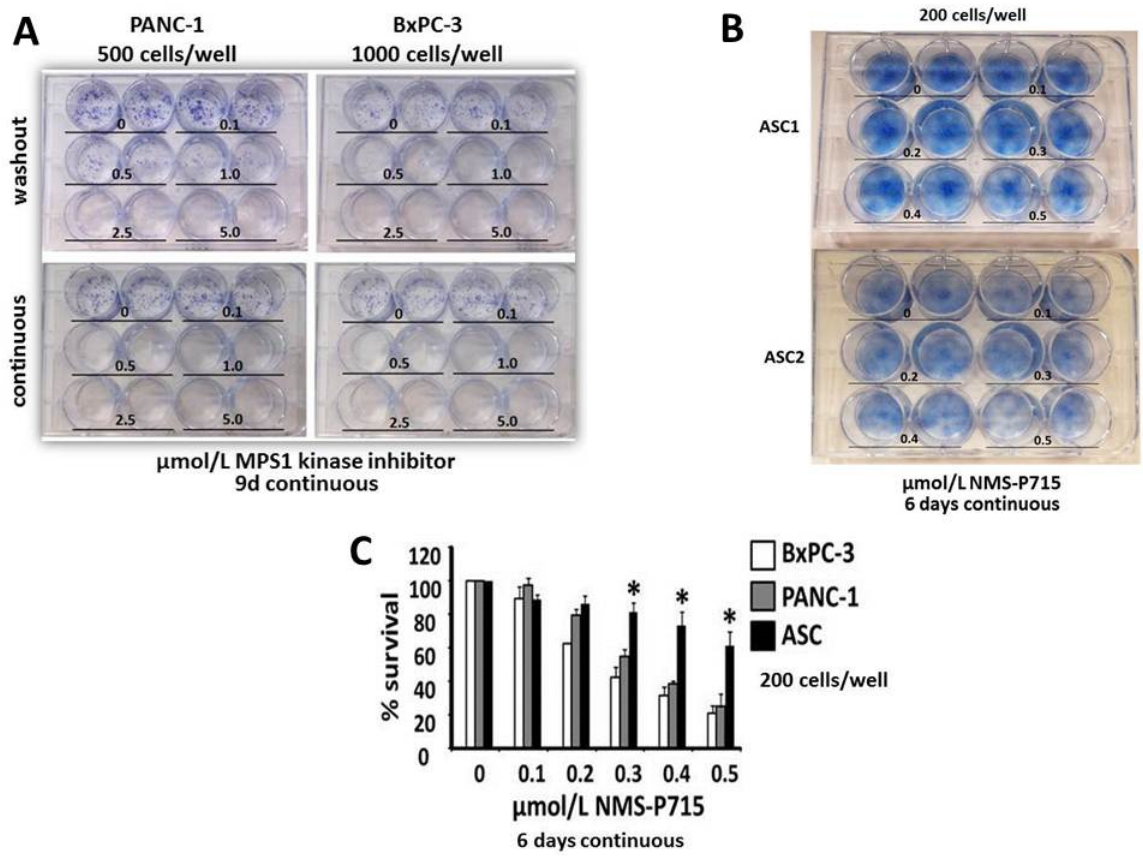

Figure 12 NMS-P715 selectively inhibits cancer cell proliferation leaving ASCs relatively unaffected

A: Growth inhibition of PANC-1 and BxPC-3 cells plated at concentrations of 500 and 1000cells/well respectively when treated with NMS-P715 for 9 days continuously or for 24 hours followed by growth in compound-free medium for 9 days (washout treatment).

B: Cell proliferation of two independent ASC1 and ASC2 isolates treated with NMS-P715 for 6 days continuously. The cells were seeded at concentration of 200cells/well.

C: Comparison of clonogenic effect in BxPC-3, PANC-1 and ASC after treatment with NMS-P715 for 6 days continuously using colorimetric analysis. The cells were plated at a density of 200cells/well.

The assays were performed in duplicates.

published in (Slee et al. 2014).

To permit additional assessment of the anti-proliferative effects of the MPS1 inhibitor, PANC-1 cells and ASCs were first treated with 1.0 µmol/L NMS-P715 for 72 hours in a different clonogenic survival assay. DMSO was used as control. Cells were harvested and plated at 400 cells/well in compound-free medium in a 12 well dish for 6 days. PANC-1 cells showed significantly higher growth inhibition (72.2%) than that observed in ASC3 (16.1%) and ASC4 (23.2%) (Figure 13) (Slee et al. 2014).

2. Pancreatic cancer cells bypass the mitotic checkpoint after treatment with NMS-P715.

The spindle assembly checkpoint (SAC) is an essential regulator of mitotic cell division where it acts as a gatekeeper during the transition of the cell from metaphase to anaphase thus keeping chromosome segregation under check (Musacchio and Salmon 2007). It delays the onset of anaphase until all kinetochores are properly attached to the spindle microtubules (Schmidt et al. 2005). Defects in this protein complex abrogate the checkpoint mechanism causing premature transition in anaphase (Thompson and Compton 2008). It has been previously shown that cells having a weak SAC show chromosomal instability (CIN) and undergo mitotic catastrophe (Burds, Lutum, and Sorger 2005). With this rationale, we wanted to assess whether MPS1 inhibition makes PDAC cells override the SAC, by testing the presence of phosphorylation at serine 10 on histone H3 (pS10H3). This histone modification is associated with chromosome

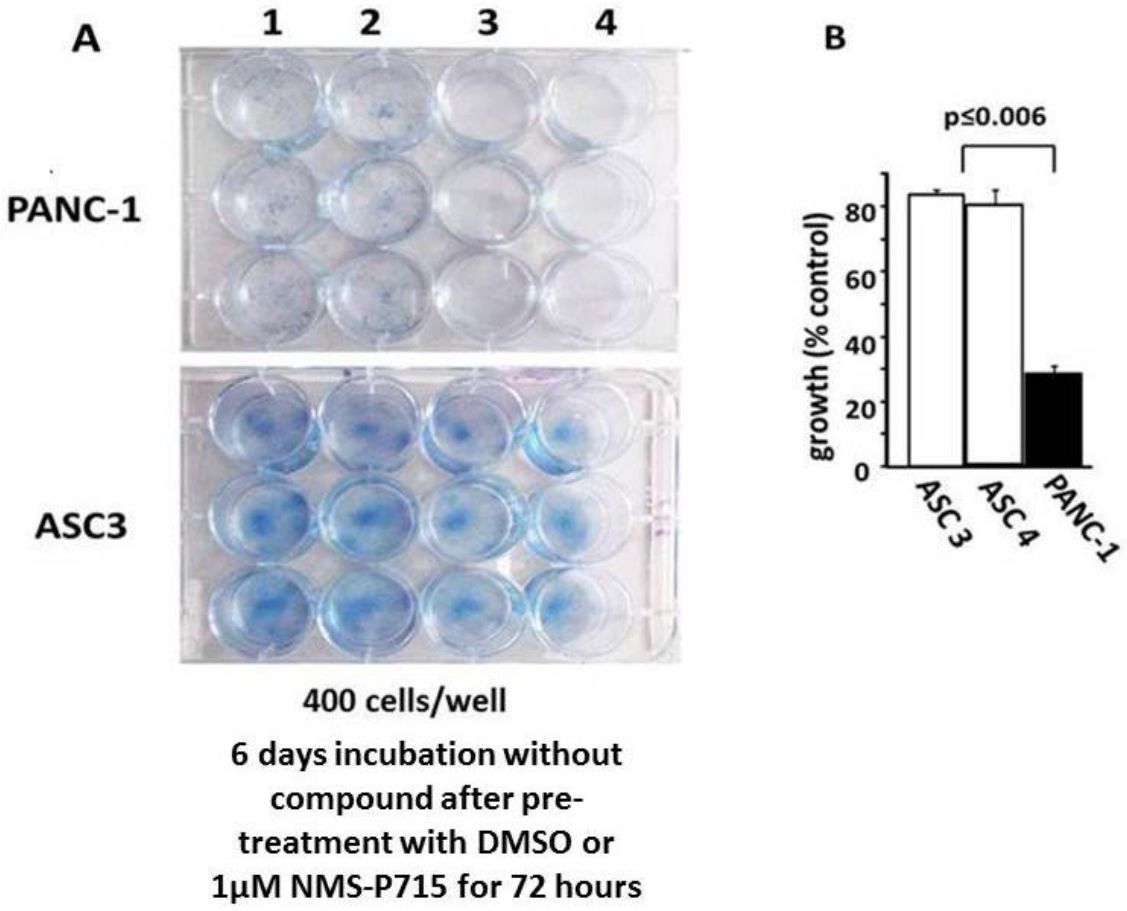

Figure 13 PDAC cell growth is inhibited by pre-treatment with 1µM NMS-P715 for 72 hours while human adipose stem cells are markedly less affected

A: Image showing PANC-1 and ASCs were pre-treated with 1µmol/L P715 for 72 hours before being plated at densities of 400cells/well in triplicate in compound free medium for 6 days. DMSO was used as control. Cells in columns 1 and 2 are controls while those in 3 and 4 are treated with the inhibitor.

B: Graph showing percent growth inhibition of PANC-1 and ASCs relative to control group after treatment with 1µmol/L P715 for 72 hours NMS-P715 determined by colorimetric assay. The growth of ASCs is almost three times more than PANC-1 after treatment with the inhibitor.

condensation during mitosis (Hendzel et al. 1997, Prigent and Dimitrov 2003). Phosphorylation at Ser 10 begins in late G2 phase and reaches its maximum at metaphase. Dephosphorylation begins in anaphase and ends in telophase indicating decondensation of chromosomes (Hendzel et al. 1997). Thus, disappearance of pS10H3 indicates that cells have passed through mitosis (Kwiatkowski et al. 2010) and we tested the absence of this marker in PANC-1 cells after treatment with NMS-P715. In addition, we repeated this experiment in ASCs to compare the effect on SAC in PANC-1 with that observed in the stem cells.

Cells were treated with increasing concentration of MPS1 inhibitor for 72 hours. The PANC-1 cells showed dose-dependent reduction of pS10H3 using Western analysis, suggesting early exit from mitosis and hence, bypassing the SAC. On the other hand, ASCs did not show a decrease in pS10H3 marker when treated with the same conditions (Figure 14).

To confirm the failure of SAC to arrest in PDAC cells, the cells were treated with NMS-P715 in the presence of nocodazole, a microtubule depolymerizing agent. Addition of nocodazole to dividing cells prevents the microtubules from attaching to the kinetochore which results in the activation of the SAC (Stucke et al. 2002).

However, cells with a weakened checkpoint will fail to arrest in prometaphase in the event of unattached kinetochores and transition into anaphase (Jelluma, Brenkman, McLeod, et al. 2008). To check the ability of NMS-P715 to cause the bypass of the

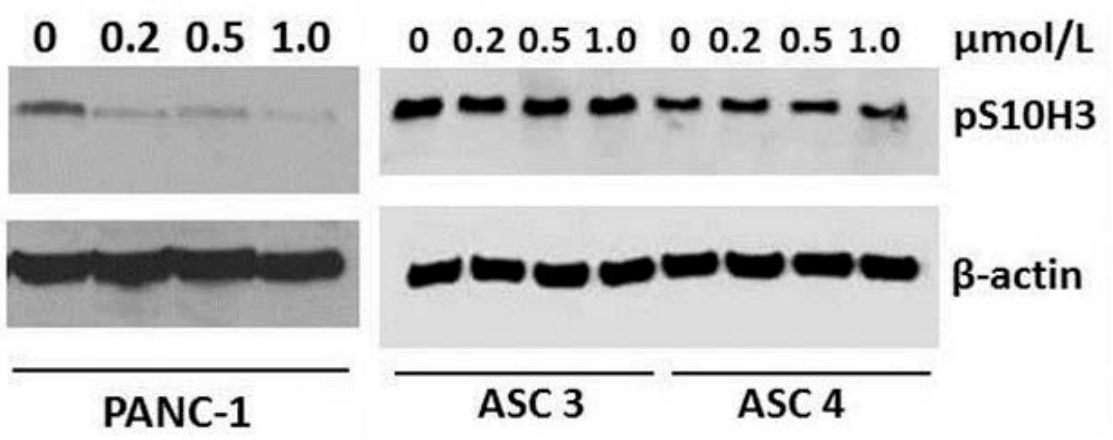

Figure 14 PANC-1 cells exhibit bypass of the SAC in the presence of NMS-P715, in contrast to ASCs which maintained an intact SAC under similar treatment conditions

Image showing western blot analysis of PANC-1 cells and ASCs when treated with the indicated concentrations of NMS-P715. '0' indicates DMSO control. Antibody to pS10H3 detected cells in mitosis while antibody to β-actin was used as control.

checkpoint, we tested the inhibitor in PANC-1 and BxPC-3 cells after activating the checkpoint signaling with nocodazole and scored the frequency of pS10H3 positive nuclei. Cells were blocked in 75nmol/L nocodazole for 18 hours and were treated with 0.4 μmol/L NMS-P715 in the last two hours of the nocodazole treatment. Both PANC-1 and BxPC-3 showed a significant decrease in pS10H3 positive nuclei after treatment with the MPS1 inhibitor (Figure 15). The reduction in the number of cells arrested in prometaphase indicates that the depletion of MPS1 in the cells caused the checkpoint to become dysfunctional resulting the cells to exit mitosis prematurely. The experiment was attempted in the ASCs. However ASCs did not grow uniformly making analysis and interpretation ambiguous. Thus we relied on results from Western analysis to measure comparative resistance to SAC override in ASCs as outlined above.

These results suggest that NMS-P715 abrogates the function of the SAC in PANC-1 cells and that ASCs are resistant to this weakening of the checkpoint when treated with same concentration of the inhibitor, thereby lending support to the possibility that the inhibitor is more selective to cancer cells than stem cells.

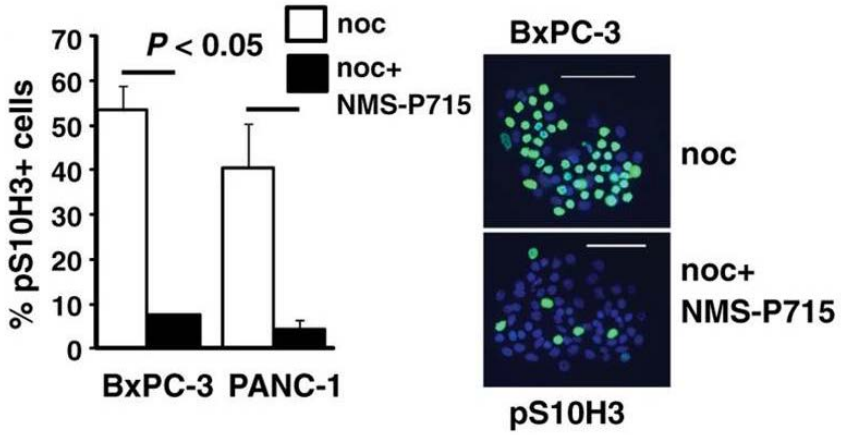

Figure 15 PDAC cells fail to arrest in prometaphase after treatment with nocodazole in the presence of NMS-P715 and exit mitosis prematurely

On the left is the graph showing the percentage of pS10H3 positive green cells when treated with nocodazole (noc) only or with nocodazole and NMS-P715 (noc + NMS-P715). A minimum of 200 cells per chamber were scored for the presence of green cells indicating the presence of pS10H3. On the right are representative images of cells when treated with noc or noc + NMS-P715. Nuclei are stained with DAPI (blue). The assay was performed in duplicate.

3. Pancreatic cancer cells are more chromosomally unstable than normal healthy cells after treatment with NMS-P715.

A decrease in the activity of MPS1 abrogates the functioning of the SAC (Jelluma, Brenkman, McLeod, et al. 2008) and increases errors in chromosomal segregation thereby promoting catastrophic CIN (Kops, Foltz, and Cleveland 2004, Colombo and Moll 2010). In addition, elevation of CIN beyond a threshold is not tolerated by cancer cells and they succumb to the deleterious effects of this phenotype (Cahill et al. 1999, Kops, Foltz, and Cleveland 2004) making CIN elevation an attractive therapeutic target in the treatment of many cancers (Colombo et al. 2010). With this rationale, we wanted to test for increased levels of numerical CIN in PDAC after treatment with the MPS1 inhibitor. We also wanted to check whether ASCs and hTERT-HPNE were resistant to increase in nCIN under conditions similar to that in PANC-1, suggesting a therapeutic window exists allowing selective targeting of cancer cells. To explore this hypothesis, we performed fluorescence *in situ* hybridization (FISH) to examine levels of nCIN after treating the cells with 1 µmol/L NMS-P715 or DMSO for 72 hours by assessing changes in percent modal deviation (%MD) for chromosome enumeration probes after treatment which is an accepted indirect measure of the CIN rate. %MD was calculated for chromosomes X and 17. As the alpha satellite DNA is divergent between centromeres in humans, probes were specific to chromosomes X and 17 (Willard 1985).

PANC-1 showed significantly elevated levels of nCIN as compared to ASCs prior to treatment, consistent with the chromosomally unstable phenotype of cancer cells (Figure

16). PANC-1 showed a dramatic increase in nCIN from 13% MD for chromosome X and 14% MD for chromosome 17 when treated with DMSO to 63% MD for chromosome X and 59% for chromosome 17 after treatment with 1 µmol/L inhibitor for 72 hours. On the other hand, the %MD in ASCs was less than 5% in both control and test samples making the change after treatment with NMS-P715 statistically insignificant suggesting that the chromosome mis-segregation rate was similar in treated or control conditions. To understand the limit to which the ASCs can tolerate the drug, we treated the cells with 3 µmol/L NMS-P715 for 72 hours and observed a significant, but modest increase in %MD from less than 5% for chromosomes X and 17 in control to 20% for chromosome X and 17% for chromosome 17 after treatment with the higher concentration of the drug.

In addition, normal pancreatic epithelial cells (hTERT-HPNE), which are telomerase-immortalized, showed a small but statistically insignificant increase in nCIN after treatment with 1 µmol/L NMS-P715 for 72 hours providing further supporting evidence that untransformed cells are less susceptible to SAC over-ride using an MPS1 inhibitor (Figure 17). On the contrary, a statistically significant increase in nCIN was observed in PANC-1 cells when they were treated under similar conditions as hTERT-HPNE.

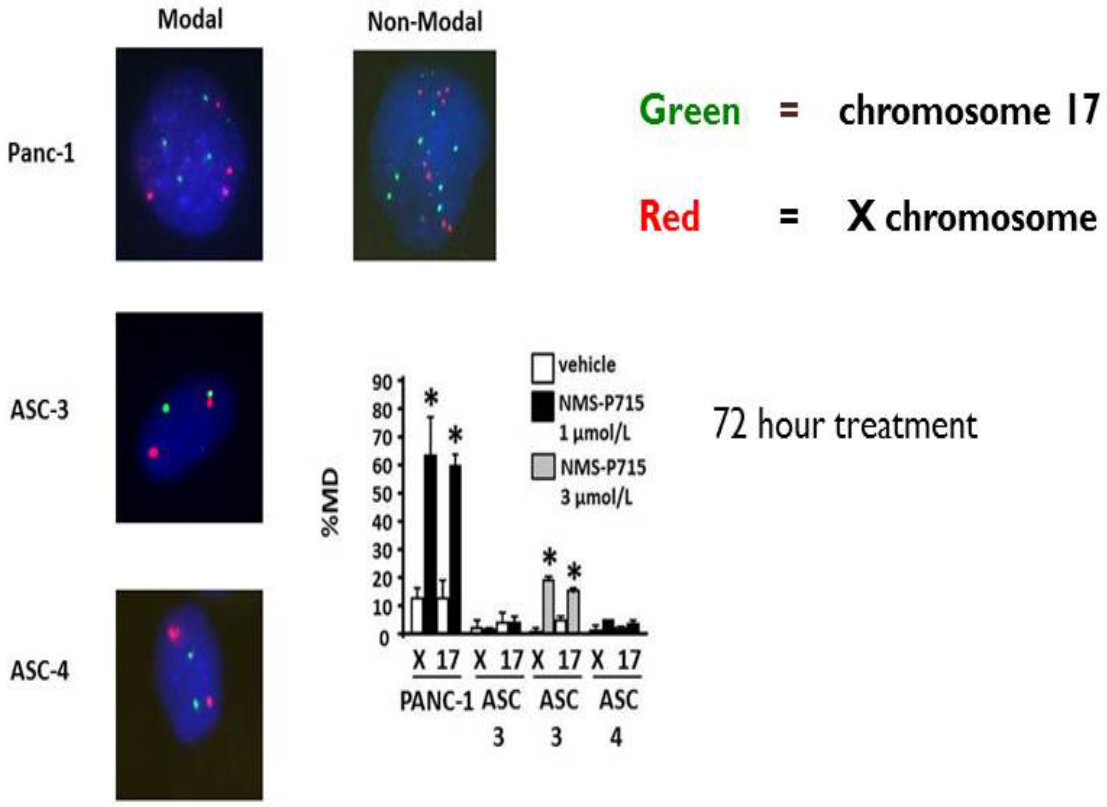

Figure 16 MPS1 inhibition elevates nCIN in PANC-1 cells but not in ASCs

PANC-1 and ASCs were treated with 1 µmol/L NMS-P715 for 72 hours and processed for FISH. ≥50 cells per culture were analyzed for the number of chromosomes. Green probe represents chromosome 17 whereas red indicates chromosome X. The modal number for chromosomes 17 and X was 4 for PANC-1 and 2 for ASCs. PANC-1 cells show significant elevation in CIN after treatment with the inhibitor. Although ASCs are resistant to the inhibition of MPS1 under conditions similar to that for PANC-1, the stem cells show significant but modest increase when treated with higher concentration (3 µmol/L) of the drug. The assay was performed in 2 replicate plates per treatment group.

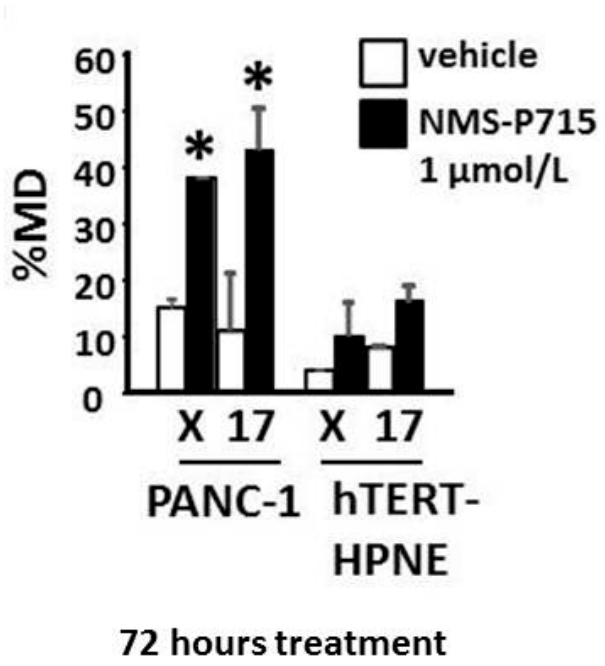

Figure 17 hTERT-HPNE cells are resistant to elevation of nCIN when treated with 1 µmol/L NMS-P715 for 72 hours

PANC-1 and hTERT-HPNE cells were treated with 1 µmol/L NMS-P715 or DMSO for 72 hours and processed for FISH analysis. A minimum of 50 interphase cells per culture were scored for number of chromosomes X and 17. hTERT-HPNE show slight but statistically insignificant increase in percent modal deviation whereas PANC-1 show a dramatic increase in %MD for both chromosomes X and 17, indicating higher sensitivity towards NMS-P715 than that observed in hTERT-HPNE under similar conditions. The assay was performed in 2 replicates per treatment group.

4. Growth of murine PDAC cells is inhibited by NMS-P715

To assess the effect of MPS1 inhibition in murine PDAC cells, 825-2 and 1170-4 KRC cells were checked for their clonogenic capacity after treatment with NMS-P715. KRC cells were derived from PDAC tumors arising from genetically engineered mice with oncogenic Kras and deletion of the retinoblastoma (Rb) gene using Cre-recombinase (Carriere et al. 2011). 825-2 and 1170-4 KRC cells were plated at a density of 100 and 50 cells/well respectively and were treated with NMS-P715 at concentrations ranging from 0.3 µmol/L to 1.0 µmol/L for 5 days continuously or for 24 hours followed by growth for 4 days in the absence of the inhibitor (washout treatment). DMSO was used as vehicle control. Large colonies with ≥50 cells that were stained with giemsa were scored. There is dose depended decrease in the growth of KRC cells after inhibition of MPS1. In the continuous treatment with the drug, the growth of 825-2 and 1170-4 cells was completely inhibited at drug concentrations from 0.5-0.7 µmol/L and 0.7-0.9 µmol/L respectively whereas in the washout experiment the growth of the KRC cells decreased by 85% when treated with 1.0 µmol/L for 24 hours (Figure 18A) suggesting a very potent effect on these tumor cells which are very aggressive *in vivo*.

In addition, the murine PDAC cells were also tested for nCIN both before and after inhibition of MPS1. 825-2 and 1170-4 cells were treated with 2.4 µmol/L and 2.7 µmol/L respectively for 72 hours after which they were analyzed by FISH. The concentrations of NMS-P715 used in this experiment were in calculated in accordance to their IC_{50} values of 1.3 and 2.2 µmol/L for 825-2 and 1170-4 respectively which was determined by

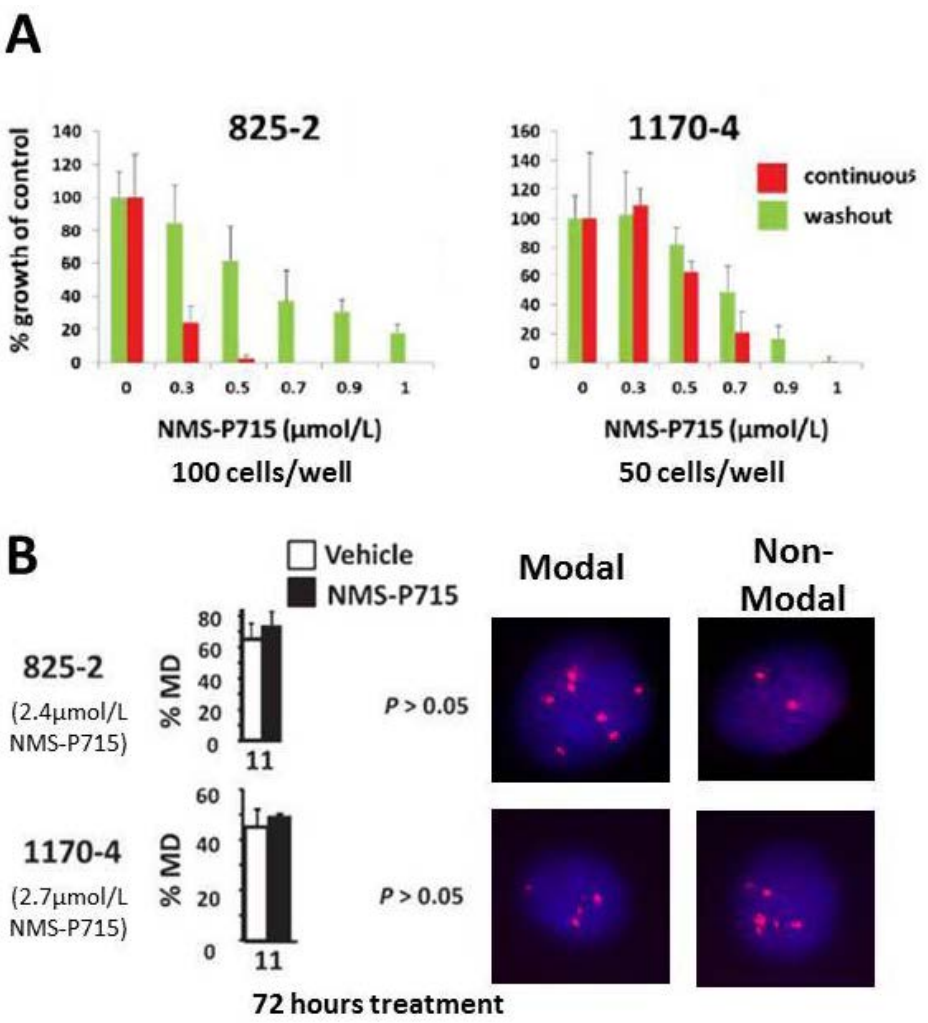

Fig 18 Growth of murine PDAC was inhibited by NMS-P715

A: Clonogenic survival assay. 825-2 and 1170-4 were seeded at a density of 100 and 50 cells/well and treated with then indicated concentrations of NMS-P715 for either 5 days in a continuous treatment or for 24 for hours followed by growth in compound-free medium in washout treatment. Colonies that had at least 50 cells stained with giemsa were scored.

B: On the left is a graph showing that nCIN in not elevated in murine PDAC cells after treating 825-2 and 1170-4 cells with 2.4 µmol/L and 2.7 µmol/L respectively for 72

hours. On the right is a representative image showing modal and non-modal number of chromosomes in the cells. The modal number of chromosome 11 was 7 for 825-2 and 4 for 1170-4.

Dr. Slee (Slee et al. 2014). The KRC cells did not show statistically significant elevation in nCIN after MPS1 was inhibited (Figure 18B). However, since KRC cells already display high nCIN with 65% and 45% modal deviation for a chromosome 11 probe in 825-2 and 1170-4 in untreated cells they start with an extremely high basal nCIN rate. Cells with deleted Rb gene, which is the case in KRC cells, have been reported to be chromosomally unstable due to misregulation of MAD2 protein via E2F transcription factor (Manning, Longworth, and Dyson 2010). The very high basal nCIN rate in KRC cells prompted us to explore whether the SAC was compromised in KRC cells. KRC cells were treated with increasing concentration of nocodazole with the rationale that a weakened SAC will bypass the checkpoint and exit mitosis prematurely as they will not be blocked by nocodazole treatment. In this experiment, cells were plated at 10,000 to 20,000 cells/well and treated with 25nmol/L, 50nmol/L and 75nmol/L nocodazole for 18 hours. DMSO was used as control. The cells were then fixed in 4% formaldehyde and incubated with pS10H3 (green) antibody. The frequency of cells positive for pS10H3 signals was calculated to test whether the cells were arrested in prometaphase when the microtubules were prevented from attaching to the kinetochore indicating the activation of SAC. There was no significant difference between the percentages of pS10H3 positive cells in control than those treated with nocodazole though the number of cells decreased with increasing concentration of nocodazole (Figure 19). However cells appeared to be less viable when incubated with nocodazole. This raises the possibility that the SAC is weak in KRC cells which reduced their viability as they try to go through an aberrant mitosis in the presence of nocodazole. This elevated sensitivity to nocodazole in cells with a weakened SAC is consistent with earlier published data (Sihn et al. 2003). Since

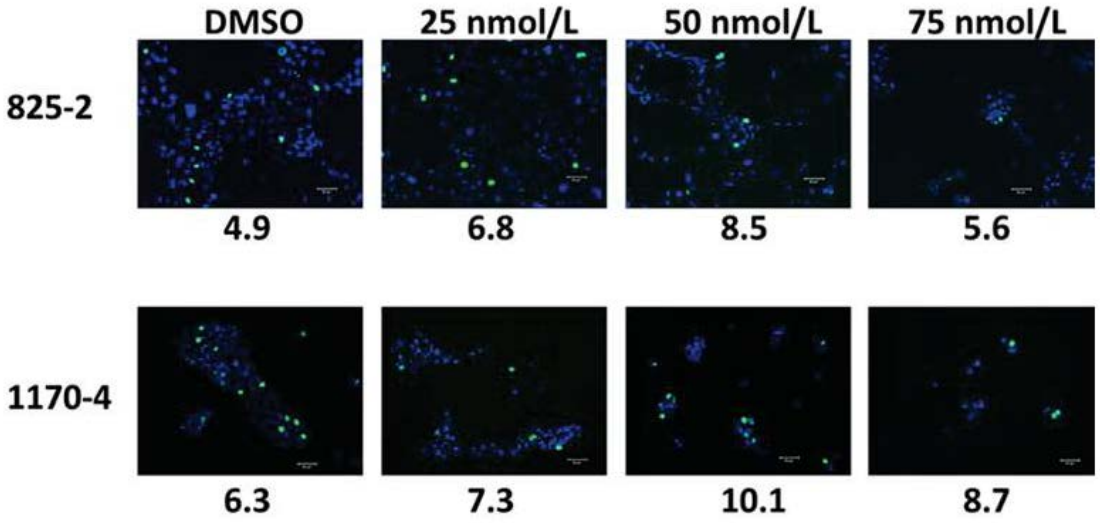

Fig 19 Murine PDAC cells may have a weakened SAC

Image shows murine PDAC cells arrested in prometaphase after 18 hour treatment with increasing concentrations of nocodazole as indicated. The percentage of cells positive for pS10H3 (green) is indicated below each panel. Nuclei appear blue due to staining with DAPI. The frequency of cells arrested in prometaphase in the treated cells was not significantly different than that observed in control ($p>0.05$; χ^2 test). ≥200 cells were scored per well. The assay was performed in duplicate.

825-2 and 1170-4 cells already showed high basal nCIN in control treatment, increasing nCIN further may be lethal.

In conclusion, the relative resistance towards NMS-P715 displayed by human ASCs and hTERT-HPNE normal pancreatic epithelial cells indicates that NMS-P715 may be selective towards pancreatic ductal adenocarcinoma cells while sparing stem cells. Mouse KRC PDAC cells, which are highly aggressive tumor cells *in vivo*, were also highly sensitive to NMS-P715 lending further support to the possibility that MPS1 inhibition may be a useful strategy for limiting growth of PDAC cells *in vivo*.

DISCUSSION

1. Thesis Summary

Pancreatic ductal adenocarcinoma (PDAC) is the only major cancer with a 5 year survival rate in the single digits i.e. 6% (Siegel, Naishadham, and Jemal 2012, Hoos et al. 2013). It is one of the commonly diagnosed cancers for which both the incidence and death rate is increasing (Hoos et al. 2013). Gemcitabine, which is the standard of care either alone or in combination with erlotinib or folfirinox (Sullivan and Kozuch 2012) can only provide a short increase in survival and has toxic effects (Choi, Saif, and Kim 2014). This has warranted the development of therapeutics that target PDAC cells, that have improved efficacy and are less toxic to normal cells.

The goal of this study was to exploit a vulnerability of PDAC cells. Like many tumors, PDAC tumors are chromosomally unstable, shows intra-tumor heterogeneity and up-regulate MPS1 (Karhu, Mahlamaki, and Kallioniemi 2006, Gorunova et al. 1998, Slee et al. 2014). We demonstrate that PDAC cells are relatively more sensitive to MPS1 inhibition than human adipose stem cells.

Exploitation of a vulnerability in cancer cells has been traditionally linked to the existence of a pre-existing mutation followed by a second hit leading to cancer cell death. This has been successfully demonstrated in BRCA1 deficient tumors using PARP inhibitors (Fong et al. 2009). Here we build upon the hypotheses being proposed in the

literature that indicate that cancer cells become addicted to up-regulation of genes involved in chromosome segregation and that targeting them may lead to selective cancer cell death via massive chromosome mis-segregation (Colombo et al. 2010, Carter et al. 2006, Yuan et al. 2006, Kwiatkowski et al. 2010, Lens, Voest, and Medema 2010, Sausville 2004). The antimitotic inhibitor of MPS1 kinase used in this study is modeled on the approach where selective increase of chromosomal instability may act as a second hit and push CIN beyond survivable limits leaving normal cells relatively unaffected (Colombo et al. 2010). Cancer cells, including pancreatic cancer cells and other solid tumors, up-regulate MPS1 and this up-regulation is proposed to keep nCIN within survivable limits (Carter et al. 2006, Yuan et al. 2006, Grabsch et al. 2003, Slee et al. 2014). Inhibiting MPS1 kinase, a protein essential in the normal and equal segregation of chromosomes during mitosis, causes the abrogation of the spindle assembly checkpoint (SAC) followed by massive CIN and eventually cell death (Colombo et al. 2010, Kops, Foltz, and Cleveland 2004, Schmidt et al. 2005, Kwiatkowski et al. 2010). In previous studies, normal untransformed cells such as fibroblasts and B-lymphocytes remain largely unaffected by the NMS-P715 relative to breast cancer, colon cancer, renal carcinoma and melanoma cells (Colombo et al. 2010). Here, we tested the selective nature of MPS1 inhibition in normal human adipose stem cells (ASCs) as a model for how stem cells of the intestine or blood may be affected *in vivo*. The goal of my dissertation was to test the hypothesis that NMS-P715 can selectively kill PDAC cells *in vitro* by elevating mis-segregation of chromosomes while having much less effect on stem cells. Our *in vitro* MPS1 kinase inhibition assays in PDAC show promising results in selectively targeting pancreatic cancer cells by overriding the SAC and increasing

chromosomal mis-segregation ultimately leading to cell death. This is consistent with the consequences of MPS1 inhibition observed over a range of cancer cell lines and xenograft models reported in previous studies (Colombo et al. 2010, Jelluma, Brenkman, McLeod, et al. 2008, Jelluma, Brenkman, van den Broek, et al. 2008, Kops, Foltz, and Cleveland 2004, Burds, Lutum, and Sorger 2005, Schmidt et al. 2005) and our studies demonstrate promise that MPS1 inhibition results in selective targeting of PDAC.

2. NMS-P715 selectively inhibits pancreatic cancer cell proliferation leaving human adipose stem cells relatively unaffected.

In previous studies done by Dr. Slee in the Grimes lab, the half maximal inhibitory concentration value of the small-molecule inhibitor, NMS-P715, in ASCs is more than twice as that observed in the pancreatic cancer cells. In contrast, ASCs were highly sensitive to gemcitabine compared to PDAC cells (Slee et al. 2014). The differences between the growth of human ASCs and cancer cells was more pronounced in longer term clonogenic survival assays, when cells were either continuously treated for 3 days with 1.0 μmol/LNMS-P715 or exposed to a one day pre-treatment with NMS-P715. The inhibitor showed higher activity toward inhibition of proliferation of PANC-1 and BxPC-3 PDAC cells while growth of ASCs was markedly less impacted. Growth of PDAC cells was inhibited when treated with 0.5 μmol/L NMS-P715 but ASCs showed only a marginal decrease in cell growth after treatment with the same concentration of the inhibitor. Being a long term assay to assess the proliferative capacity of cells after treatment, clonogenic survival assay takes into account all forms of cell death

mechanisms activated by the external insult to the cells as well as the heterogeneity within the population of cells with regards to the time of activation of the cell death machinery after treatment (Brown and Attardi 2005). The anti-proliferative results obtained in this study are in consistence with previous studies which showed that inhibition of essential mitotic checkpoint proteins such as MPS1 and BUBR1 causes death in cells within 6 cell divisions (Kops, Foltz, and Cleveland 2004). The new data presented here demonstrate that MPS1 inhibition results in significant growth impairment of PDAC cells while having less effect on ASCs thus opening up the possibility of a therapeutic window using MPS1 treatment to selectively target tumor growth *in vivo*.

3. Selective override of the SAC in PDAC cells following MPS1 inhibition

Our studies demonstrated that NMS-P715 abrogates the SAC in PDAC cells. Phosphorylation of Histone H3 at serine 10 (pS10H3) is a marker of cells in mitosis. Western analysis showed that PDAC cells exhibited a dose-dependent decrease in pS10H3 upon NMS-P715 treatment, reflecting premature exit from mitosis due to SAC over-ride whereas ASCs were unaffected under similar conditions. Furthermore NMS-P715 treatment caused over-ride of a nocodazole block, again supporting the mechanism of SAC over-ride by NMS-P715. Finally our FISH analysis of cells treated with 1 μmol/L NMS-P715 was consistent with an increase in nCIN in PDAC cells while ASCs were not affected. These data, together with the comparative increase in cell growth inhibition of PDAC cells relative to ASCs supports our hypothesis that NMS-P715 treatment caused selective death to PDAC cells via massive CIN under conditions where stem cells are less

affected. The results in ASCs were further supported by NMS-P715 treatment of telomerase immortalized pancreatic ductal epithelial cells (hTERT-HPNE-1 cells). hTERT-HPNE cells also did not exhibit growth inhibition or elevated nCIN under conditions were PDAC cells exhibited increased nCIN and cell growth inhibition, again supporting the possibility of a therapeutic window where PDAC cells could be selectively targeted by MPS1 inhibition. These studies provide new insights into the mechanism of resistance of non-tumorigenic cells to MPS1 inhibition and suggest that the SAC is more resistant to override by MPS1 inhibition in stem cells and pancreatic epithelial cells than PDAC cells. The difference observed in differential sensitivity to MPS1 inhibition of tumorigenic versus non-tumorigenic cells could be related to 1) the increased basal levels of nCIN (as measured by the FISH assay) and 2) the relative up-regulation of MPS1 in PDAC cells. Current models suggest both that the increased level of CIN (both structural and numerical) make cancer cells particularly sensitive to increases in CIN (such as shown here by MPS1 inhibition and SAC abrogation) than normal cells, such as ASCs and hTERT-HPNE cells that are diploid (Grimes et al. 2009, Slee et al. 2014) as is evident from the data presented in this thesis. Furthermore, cancer cells may be addicted to up-regulation of MPS1 for their survival. The vulnerability of cancer cells to MPS1 inhibition is discussed in further sections.

4. **Murine PDAC cells show impaired growth when treated with NMS-P715 and may have a weakened SAC**

The anti-tumor activity of NMS-P715 was elevated in murine PDAC cells that were obtained from genetically engineered mice with oncogenic Kras and deleted Rb using cre-recombinase (KRC cells 825-2 and 1170-4) (Carriere et al. 2011). NMS-P715 affected their proliferation in long-term clonogenic survival assays where the growth of 825-2 & 1170-4 was completely abolished when treated continuously for 5 days with approx. 0.7 µmol/L inhibitor whereas their growth decreased by 85% after treatment with 1.0 µmol/L for 24 hours. The KRC cells showed extreme CIN phenotype in the control group but did not show an increase in nCIN after treatment with their IC_{50} concentrations of the inhibitor for 72 hours. These data are consistent with previous reports where the inactivation of Rb gene is linked to CIN (Manning, Longworth, and Dyson 2010). Furthermore, the cells failed to arrest in prometaphase in the presence of nocodazole, a microtubule depolymerizing agent, indicating a weakened SAC (Sihn et al. 2003). As a result, the inhibition of MPS1 in murine PDAC cells may have completely abrogated the SAC making the treatment with NMS-P715 lethal to these cells (Kops, Foltz, and Cleveland 2004).

Since *in vitro* results do not always mimic *in vivo* results, it will be interesting to see the anti-tumor effects of NMS-P715 *in vivo* by transplanting KRC cells in the pancreata of syngeneic immune competent mice. It has been observed that tumors *in situ* contain significantly lower percentage of dividing cells than in cell culture (Mitchison 2012)

which may affect the efficacy of the inhibitor *in vivo* since it only attacks mitotic cells. In addition, tumors are not only genetically heterogeneous but their microenvironments show variability as well (Orth et al. 2011) causing differential response within the tumor resulting in individual cells responding differently to the same compound (Yang et al. 2010). Using syngeneic mice will allow the analysis of microenvironmental interactions, endocrine signaling tumor-secreting factors as well as effects of the immune system and vasculature on the tumor in response to the inhibitor (Pearson and Pouliot 2000). In addition, it will become possible to examine the impact of the inhibitor on the metastatic behavior of PDAC with the help of syngeneic mouse models (Pearson and Pouliot 2000). Testing these mice with NMS-P715 will give us a better understanding by taking into consideration factors such as cell-to-cell contact and hypoxia which are known to confer drug resistance *in vivo* (Sarasin 2003).

5. Inhibition of MPS1 is selective towards PDAC cells *in vitro* whereas normal cells are less affected under similar conditions.

To address the possibility that ASCs could not uptake small molecule inhibitors efficiently, an independent assay was conducted. The half minimal concentration (IC_{50}) value of NMS-P715 for ASCs and hTERT-HPNE was 3.4 µmol/L indicating that the inhibitor did have an effect on cell growth though at a concentration much higher than that required for PANC-1 and BxPC-3 (Slee et al. 2014). In addition, ASCs showed statistically significant increase in nCIN after the cells were treated with 3 µmol/L inhibitor for 72 hours suggesting that the molecule was successful in inactivating the

SAC. These results indicate that the tumor cells lack the shield which normal cells possess in order to resist the inhibitory effects of NMS-P715.

PDAC cells are aneuploid and display intra-tumor heterogeneity with respect to chromosome number as well as structure (Sirivatanauksorn et al. 2001) whereas ASCs maintain a diploid number (Grimes et al. 2009). In addition to other genetic and non-genetic factors, mitosis in aneuploid cells is usually prolonged due to extra chromosomes that have to be attached to the spindle microtubules, aligned on the metaphase plate and segregated to the daughter cells (Yang et al. 2008). In addition, chromosomally unstable cells have more stable kinetochore-microtubule (KT-MT) attachments than normal cells due to massive variations in the expression of different spindle and kinetochore proteins in dividing tumor cells (Bakhoum, Genovese, and Compton 2009). The altered expression of these proteins also results in merotelic attachments of microtubules to the kinetochores which are usually undetected by the SAC resulting in lagging chromosomes during anaphase further triggering chromosomal mis-segregation (Bakhoum, Genovese, and Compton 2009). Inhibition of MPS1 is known to stabilize the already hyperstable KT-MT attachment (Kwiatkowski et al. 2010) thereby affecting segregation of chromosomes.

Because normal cells differ from most tumor cells with respect to the number of chromosomes, stability of KT-MT attachments and balance of gene doses, MPS1 inhibition can potentially be more selective towards PDAC cells. This increased sensitivity could be due to extensive genetic imbalances that the cancer cells had started

off with before treatment which make them unable to repair the damage and combat the stress that accompanied MPS1 inhibition (Kops, Weaver, and Cleveland 2005, Williams et al. 2008). Though our data demonstrates that NMS-P715 significantly affects ASCs at higher doses, the effect may be minimal and the affected cells may have been removed from the population as a result of selection towards diploid cells. However, the possibility of tumorigenic mutations in ASCs after treatment needs full considerations and will require further analysis of long term effects of the inhibitor on healthy cells.

6. Future Directions

The results of this study show great promise in selective killing of pancreatic cancer cells by inhibiting MPS1 kinase using a single-agent NMS-P715. However, in *vivo* analyses for anticancer drugs are necessary to understand the efficacy and delivery of the molecule in the target region of pancreatic cancer. This can be achieved by testing the inhibitor in orthotopic implantation of human PDAC cell lines in immunocompromised mice and also in syngeneic mouse models of pancreatic cancer as discussed earlier (Carriere et al. 2011). Tumor microenvironment plays an important role in drug resistance, tumor recurrence as well as drug delivery (Olive et al. 2009). PDAC in mice, just as in human PDAC, is surrounded by a dense matrix that limits the blood supply to the malignant cells by compressing the vasculature thereby resulting in poor drug uptake and efficacy (Hingorani et al. 2003, Sofuni et al. 2005) making it necessary to study the inhibitor *in vivo* to improve its delivery to its site of action. It was earlier shown that MPS1 inhibition in xenograft mouse models for human ovarian carcinoma and melanoma impeded tumor

growth (Colombo et al. 2010) and it now remains to be seen if these results are replicated in mouse models for PDAC as well.

One of the biggest challenges in cancer therapeutics is tumors becoming drug-resistant. According to the National Cancer Institute, therapeutic resistance is a characteristic feature of PDAC (NCI 2014). Cancer cells become insensitive to therapeutic agents by different mechanisms such as accumulating mutations that enhance the cells' survival, acidic microenvironment or limited blood supply to the tumor cells (Olive et al. 2009). SAC components are not mutated frequently but are instead overexpressed in chromosomally unstable tumors (Wang et al. 2004, Cahill et al. 1999, Carter et al. 2006, Yuan et al. 2006, Slee et al. 2014). However an unstable genome can give rise to new mutations that alter proteins and make them resistant to anti-cancer drugs. A study in 2005 showed that a substitution mutation from methionine to glutamine in MPS1 kinase (M602Q) made the cells resistant to its inhibitor SP600125 thereby reducing its potency (Schmidt et al. 2005). This calls for testing the efficacy of NMS-P715 in PDAC cells in the event of an M602Q mutation or other similar alterations in the protein.

To overcome the problem of drug-resistance, there has been more focus on designing combination therapies to improve patient outcomes. Various studies are being carried out to determine whether cancer cells can be sensitized to MPS1 inhibition by combination with compounds targeting other mechanisms critical for cells survival pathways that rescue cells from proteotoxic stress and proteins involved in DNA damage response (Torres et al. 2007, Janssen, Kops, and Medema 2009). It has been previously shown that

reducing the levels of MPS1 increased the sensitivity of tumor cells to low doses of paclitaxel, a microtubule destabilizing agent, resulting in synergistic lethality of the treated cells. (Janssen, Kops, and Medema 2009). Interestingly, both nab-paclitaxel (Frese et al. 2012) or IPI-926, the inhibitor of the Hedgehog signaling pathway (Olive et al. 2009), are individually shown to improve the delivery of gemcitabine in PDAC in mice which may also improve NMS-P715 delivery to tumors in future studies. Enhancing multipolar division by preventing clustering of chromosomes along with MPS1 inhibition is also known to be a promising strategy in dramatically reducing the viability of PDAC tumor cells (Kwon et al. 2008, Janssen and Medema 2011). Furthermore, the ability to selectively reduce chromosomally unstable cells within the tumor may give agents targeting pathways altered in patient sub-populations more chance of working as tumor heterogeneity that is thought to promote drug resistance would be suppressed due to MPS1 inhibition.

To ensure clinical success of NMS-P715 in the treatment of PDAC, a long term endpoint in clinical trials is needed to determine whether MPS1 inhibition could promote cancer in healthy cells if, at a very low level, CIN is increased through a minor effect on the SAC. As with any drug, it will be necessary to study whether NMS-P715 shows good tumor penetration and efficacy and exhibit toxicity within acceptable limits. At least in xenografts for ovarian cancer and malignant melanoma, NMS-P715 showed good efficacy and target engagement and no overt toxicities were reported (Colombo et al. 2010). Interestingly, a derivative potent and selective MPS1 kinase inhibitor developed by Nerviano Medical Sciences will soon enter Phase I clinical trials in triple-negative

breast cancer (TNBC) patients (NMS 2013) which lack over-expression of human epidermal growth factor 2 (HER2) and absence or reduced expression of progesterone estrogen receptors. TNBC cells are also the most chromosomally unstable subset of breast cancers (Smid et al. 2011, Foulkes, Smith, and Reis-Filho 2010). It will be interesting to follow the results of these clinical trials in the hope that they will yield positive results without serious side effects and their subsequent testing in other tumor types, including pancreatic cancer.

7. Concluding Remarks

The data presented in this study lead us to conclude that the abrogation of the SAC by inhibiting MPS1 kinase is a potentially novel approach to pancreatic cancer therapy because it selectively blocks the proliferation of pancreatic tumor cells while leaving normal stem cells relatively unaffected. Our results suggest a favorable therapeutic window of the MPS1 inhibitor and warrant development of pre-clinical models for testing the capacity of NMS-P715 to selectively target primary and metastatic PDAC tumors.

REFERENCES

Bakhoum, S. F., and D. A. Compton. 2012. "Chromosomal instability and cancer: a complex relationship with therapeutic potential." *J Clin Invest* no. 122 (4):1138-43. doi: 10.1172/jci59954.

Bakhoum, S. F., G. Genovese, and D. A. Compton. 2009. "Deviant kinetochore microtubule dynamics underlie chromosomal instability." *Curr Biol* no. 19 (22):1937-42. doi: 10.1016/j.cub.2009.09.055.

Birkbak, N. J., A. C. Eklund, Q. Li, S. E. McClelland, D. Endesfelder, P. Tan, I. B. Tan, A. L. Richardson, Z. Szallasi, and C. Swanton. 2011. "Paradoxical relationship between chromosomal instability and survival outcome in cancer." *Cancer Res* no. 71 (10):3447-52. doi: 10.1158/0008-5472.can-10-3667.

Brown, J. M., and L. D. Attardi. 2005. "The role of apoptosis in cancer development and treatment response." *Nat Rev Cancer* no. 5 (3):231-7. doi: 10.1038/nrc1560.

Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. 2002. "Molecular Biology of the Cell." In. New York: Garland Science. http://www.ncbi.nlm.nih.gov/books/NBK21054/ (accessed 9.3.2014).

Burds, A. A., A. S. Lutum, and P. K. Sorger. 2005. "Generating chromosome instability through the simultaneous deletion of Mad2 and p53." *Proc Natl Acad Sci U S A* no. 102 (32):11296-301. doi: 10.1073/pnas.0505053102.

Burgess, A., M. Rasouli, and S. Rogers. 2014. "Stressing mitosis to death." *Front Oncol* no. 4:140. doi: 10.3389/fonc.2014.00140.

Cahill, D. P., K. W. Kinzler, B. Vogelstein, and C. Lengauer. 1999. "Genetic instability and darwinian selection in tumours." *Trends Cell Biol* no. 9 (12):M57-60.

Carriere, C., A. J. Gore, A. M. Norris, J. R. Gunn, A. L. Young, D. S. Longnecker, and M. Korc. 2011. "Deletion of Rb accelerates pancreatic carcinogenesis by oncogenic Kras and impairs senescence in premalignant lesions." *Gastroenterology* no. 141 (3):1091-101. doi: 10.1053/j.gastro.2011.05.041.

Carter, S. L., A. C. Eklund, I. S. Kohane, L. N. Harris, and Z. Szallasi. 2006. "A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers." *Nat Genet* no. 38 (9):1043-8. doi: 10.1038/ng1861.

Castedo, M., J. L. Perfettini, T. Roumier, K. Andreau, R. Medema, and G. Kroemer. 2004. "Cell death by mitotic catastrophe: a molecular definition." *Oncogene* no. 23 (16):2825-37. doi: 10.1038/sj.onc.1207528.

Choi, M., M. W. Saif, and R. Kim. 2014. "Is there a role for second line therapy in advanced pancreatic cancer?" *JOP* no. 15 (2):106-9. doi: 10.6092/1590-8577/2325.

Colombo, R., M. Caldarelli, M. Mennecozzi, M. L. Giorgini, F. Sola, P. Cappella, C. Perrera, S. R. Depaolini, L. Rusconi, U. Cucchi, N. Avanzi, J. A. Bertrand, R. T. Bossi, E. Pesenti, A. Galvani, A. Isacchi, F. Colotta, D. Donati, and J. Moll. 2010. "Targeting the mitotic checkpoint for cancer therapy with NMS-P715, an inhibitor of MPS1 kinase." *Cancer Res* no. 70 (24):10255-64. doi: 10.1158/0008-5472.CAN-10-2101.

Colombo, R., and J. Moll. 2010. "Destabilizing aneuploidy by targeting cell cycle and mitotic checkpoint proteins in cancer cells." *Curr Drug Targets* no. 11 (10):1325-35.

Crasta, K., N. J. Ganem, R. Dagher, A. B. Lantermann, E. V. Ivanova, Y. Pan, L. Nezi, A. Protopopov, D. Chowdhury, and D. Pellman. 2012. "DNA breaks and chromosome pulverization from errors in mitosis." *Nature* no. 482 (7383):53-8. doi: 10.1038/nature10802.

Dorer, R. K., S. Zhong, J. A. Tallarico, W. H. Wong, T. J. Mitchison, and A. W. Murray. 2005. "A small-molecule inhibitor of Mps1 blocks the spindle-checkpoint response to a lack of tension on mitotic chromosomes." *Curr Biol* no. 15 (11):1070-6. doi: 10.1016/j.cub.2005.05.020.

Fong, P. C., D. S. Boss, T. A. Yap, A. Tutt, P. Wu, M. Mergui-Roelvink, P. Mortimer, H. Swaisland, A. Lau, M. J. O'Connor, A. Ashworth, J. Carmichael, S. B. Kaye, J. H. Schellens, and J. S. de Bono. 2009. "Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers." *N Engl J Med* no. 361 (2):123-34. doi: 10.1056/NEJMoa0900212.

Foulkes, W. D., I. E. Smith, and J. S. Reis-Filho. 2010. "Triple-negative breast cancer." *N Engl J Med* no. 363 (20):1938-48. doi: 10.1056/NEJMra1001389.

Frese, K. K., A. Neesse, N. Cook, T. E. Bapiro, M. P. Lolkema, D. I. Jodrell, and D. A. Tuveson. 2012. "nab-Paclitaxel potentiates gemcitabine activity by reducing cytidine deaminase levels in a mouse model of pancreatic cancer." *Cancer Discov* no. 2 (3):260-9. doi: 10.1158/2159-8290.cd-11-0242.

Geigl, J. B., A. C. Obenauf, T. Schwarzbraun, and M. R. Speicher. 2008. "Defining 'chromosomal instability'." *Trends Genet* no. 24 (2):64-9. doi: 10.1016/j.tig.2007.11.006.

Gisselsson, D., L. Pettersson, M. Hoglund, M. Heidenblad, L. Gorunova, J. Wiegant, F. Mertens, P. Dal Cin, F. Mitelman, and N. Mandahl. 2000. "Chromosomal breakage-fusion-bridge events cause genetic intratumor heterogeneity." *Proc Natl Acad Sci U S A* no. 97 (10):5357-62. doi: 10.1073/pnas.090013497.

Gordon, D. J., B. Resio, and D. Pellman. 2012. "Causes and consequences of aneuploidy in cancer." *Nat Rev Genet* no. 13 (3):189-203. doi: 10.1038/nrg3123.

Gorunova, L., M. Hoglund, A. Andren-Sandberg, S. Dawiskiba, Y. Jin, F. Mitelman, and B. Johansson. 1998. "Cytogenetic analysis of pancreatic carcinomas: intratumor heterogeneity and nonrandom pattern of chromosome aberrations." *Genes Chromosomes Cancer* no. 23 (2):81-99.

Grabsch, H., S. Takeno, W. J. Parsons, N. Pomjanski, A. Boecking, H. E. Gabbert, and W. Mueller. 2003. "Overexpression of the mitotic checkpoint genes BUB1, BUBR1, and BUB3 in gastric cancer--association with tumour cell proliferation." *J Pathol* no. 200 (1):16-22. doi: 10.1002/path.1324.

Grimes, B. R., C. M. Steiner, S. Merfeld-Clauss, D. O. Traktuev, D. Smith, A. Reese, A. M. Breman, V. C. Thurston, G. H. Vance, B. H. Johnstone, R. B. Slee, and K. L. March. 2009. "Interphase FISH demonstrates that human adipose stromal cells maintain a high level of genomic stability in long-term culture." *Stem Cells Dev* no. 18 (5):717-24. doi: 10.1089/scd.2008.0255.

Hansel, D. E., S. E. Kern, and R. H. Hruban. 2003. "Molecular pathogenesis of pancreatic cancer." *Annu Rev Genomics Hum Genet* no. 4:237-56. doi: 10.1146/annurev.genom.4.070802.110341.

Hendzel, M. J., Y. Wei, M. A. Mancini, A. Van Hooser, T. Ranalli, B. R. Brinkley, D. P. Bazett-Jones, and C. D. Allis. 1997. "Mitosis-specific phosphorylation of histone H3 initiates primarily within pericentromeric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation." *Chromosoma* no. 106 (6):348-60.

Hezel, A. F., A. C. Kimmelman, B. Z. Stanger, N. Bardeesy, and R. A. Depinho. 2006. "Genetics and biology of pancreatic ductal adenocarcinoma." *Genes Dev* no. 20 (10):1218-49. doi: 10.1101/gad.1415606.

Hingorani, S. R., E. F. Petricoin, A. Maitra, V. Rajapakse, C. King, M. A. Jacobetz, S. Ross, T. P. Conrads, T. D. Veenstra, B. A. Hitt, Y. Kawaguchi, D. Johann, L. A. Liotta, H. C. Crawford, M. E. Putt, T. Jacks, C. V. Wright, R. H. Hruban, A. M. Lowy, and D. A. Tuveson. 2003. "Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse." *Cancer Cell* no. 4 (6):437-50.

Hong, S. J., D. O. Traktuev, and K. L. March. 2010. "Therapeutic potential of adipose-derived stem cells in vascular growth and tissue repair." *Curr Opin Organ Transplant* no. 15 (1):86-91. doi: 10.1097/MOT.0b013e328334f074.

Hoos, W. A., P. M. James, L. Rahib, A. W. Talley, J. M. Fleshman, and L. M. Matrisian. 2013. "Pancreatic cancer clinical trials and accrual in the United States." *J Clin Oncol* no. 31 (27):3432-8. doi: 10.1200/jco.2013.49.4823.

Janssen, A., G. J. Kops, and R. H. Medema. 2009. "Elevating the frequency of chromosome mis-segregation as a strategy to kill tumor cells." *Proc Natl Acad Sci U S A* no. 106 (45):19108-13. doi: 10.1073/pnas.0904343106.

Janssen, A., and R. H. Medema. 2011. "Mitosis as an anti-cancer target." *Oncogene* no. 30 (25):2799-809. doi: 10.1038/onc.2011.30.

Janssen, A., M. van der Burg, K. Szuhai, G. J. Kops, and R. H. Medema. 2011. "Chromosome segregation errors as a cause of DNA damage and structural chromosome aberrations." *Science* no. 333 (6051):1895-8. doi: 10.1126/science.1210214.

Jelluma, N., A. B. Brenkman, I. McLeod, J. R. Yates, 3rd, D. W. Cleveland, R. H. Medema, and G. J. Kops. 2008. "Chromosomal instability by inefficient Mps1 auto-activation due to a weakened mitotic checkpoint and lagging chromosomes." *PLoS One* no. 3 (6):e2415. doi: 10.1371/journal.pone.0002415.

Jelluma, N., A. B. Brenkman, N. J. van den Broek, C. W. Cruijsen, M. H. van Osch, S. M. Lens, R. H. Medema, and G. J. Kops. 2008. "Mps1 phosphorylates Borealin to control Aurora B activity and chromosome alignment." *Cell* no. 132 (2):233-46. doi: 10.1016/j.cell.2007.11.046.

Jemaa, M., L. Galluzzi, O. Kepp, A. Boileve, D. Lissa, L. Senovilla, F. Harper, G. Pierron, F. Berardinelli, A. Antoccia, M. Castedo, I. Vitale, and G. Kroemer. 2012. "Preferential killing of p53-deficient cancer cells by reversine." *Cell Cycle* no. 11 (11):2149-58. doi: 10.4161/cc.20621.

Kaelin, W. G., Jr. 2005. "The concept of synthetic lethality in the context of anticancer therapy." *Nat Rev Cancer* no. 5 (9):689-98. doi: 10.1038/nrc1691.

Kang, J., Y. Chen, Y. Zhao, and H. Yu. 2007. "Autophosphorylation-dependent activation of human Mps1 is required for the spindle checkpoint." *Proc Natl Acad Sci U S A* no. 104 (51):20232-7. doi: 10.1073/pnas.0710519105.

Karhu, R., E. Mahlamaki, and A. Kallioniemi. 2006. "Pancreatic adenocarcinoma -- genetic portrait from chromosomes to microarrays." *Genes Chromosomes Cancer* no. 45 (8):721-30. doi: 10.1002/gcc.20337.

Kops, G. J., D. R. Foltz, and D. W. Cleveland. 2004. "Lethality to human cancer cells through massive chromosome loss by inhibition of the mitotic checkpoint." *Proc Natl Acad Sci U S A* no. 101 (23):8699-704. doi: 10.1073/pnas.0401142101.

Kops, G. J., B. A. Weaver, and D. W. Cleveland. 2005. "On the road to cancer: aneuploidy and the mitotic checkpoint." *Nat Rev Cancer* no. 5 (10):773-85. doi: 10.1038/nrc1714.

Kwiatkowski, N., N. Jelluma, P. Filippakopoulos, M. Soundararajan, M. S. Manak, M. Kwon, H. G. Choi, T. Sim, Q. L. Deveraux, S. Rottmann, D. Pellman, J. V. Shah, G. J. Kops, S. Knapp, and N. S. Gray. 2010. "Small-molecule kinase inhibitors provide insight into Mps1 cell cycle function." *Nat Chem Biol* no. 6 (5):359-68. doi: 10.1038/nchembio.345.

Kwon, M., S. A. Godinho, N. S. Chandhok, N. J. Ganem, A. Azioune, M. Thery, and D. Pellman. 2008. "Mechanisms to suppress multipolar divisions in cancer cells with extra centrosomes." *Genes Dev* no. 22 (16):2189-203. doi: 10.1101/gad.1700908.

Lengauer, C., K. W. Kinzler, and B. Vogelstein. 1997. "Genetic instability in colorectal cancers." *Nature* no. 386 (6625):623-7. doi: 10.1038/386623a0.

Lens, S. M., E. E. Voest, and R. H. Medema. 2010. "Shared and separate functions of polo-like kinases and aurora kinases in cancer." *Nat Rev Cancer* no. 10 (12):825-41. doi: 10.1038/nrc2964.

Liu, S. T., J. B. Rattner, S. A. Jablonski, and T. J. Yen. 2006. "Mapping the assembly pathways that specify formation of the trilaminar kinetochore plates in human cells." *J Cell Biol* no. 175 (1):41-53. doi: 10.1083/jcb.200606020.

Luo, J., N. L. Solimini, and S. J. Elledge. 2009. "Principles of cancer therapy: oncogene and non-oncogene addiction." *Cell* no. 136 (5):823-37. doi: 10.1016/j.cell.2009.02.024.

Manning, A. L., M. S. Longworth, and N. J. Dyson. 2010. "Loss of pRB causes centromere dysfunction and chromosomal instability." *Genes Dev* no. 24 (13):1364-76. doi: 10.1101/gad.1917310.

McGranahan, N., R. A. Burrell, D. Endesfelder, M. R. Novelli, and C. Swanton. 2012. "Cancer chromosomal instability: therapeutic and diagnostic challenges." *EMBO Rep* no. 13 (6):528-38. doi: 10.1038/embor.2012.61.

Mitchison, T. J. 2012. "The proliferation rate paradox in antimitotic chemotherapy." *Mol Biol Cell* no. 23 (1):1-6. doi: 10.1091/mbc.E10-04-0335.

Musacchio, A., and E. D. Salmon. 2007. "The spindle-assembly checkpoint in space and time." *Nat Rev Mol Cell Biol* no. 8 (5):379-93. doi: 10.1038/nrm2163.

NCI. *"Scientific Framework for Pancreatic Ductal Adenocarcinoma (PDAC)"*. National Cancer Institute 2014. Available from http://deainfo.nci.nih.gov/advisory/ctac/workgroup/pc/pdacframework.pdf.

NMS. 2013. "Nerviano Medical Sciences and Servier Announce a Collaboration and License Agreement for a Novel Anticancer Drug". http://www.nervianoms.com/en/nms-company-en/news/archive/298-nerviano-medical-sciences-and-servier-announce-a-collaboration-and-license-agreement-for-a-novel-anticancer-drug.html.

Olive, K. P., M. A. Jacobetz, C. J. Davidson, A. Gopinathan, D. McIntyre, D. Honess, B. Madhu, M. A. Goldgraben, M. E. Caldwell, D. Allard, K. K. Frese, G. Denicola, C. Feig, C. Combs, S. P. Winter, H. Ireland-Zecchini, S. Reichelt, W. J. Howat, A. Chang, M. Dhara, L. Wang, F. Ruckert, R. Grutzmann, C. Pilarsky, K. Izeradjene, S. R. Hingorani, P. Huang, S. E. Davies, W. Plunkett, M. Egorin, R. H. Hruban, N. Whitebread, K. McGovern, J. Adams, C. Iacobuzio-Donahue, J. Griffiths, and D. A. Tuveson. 2009. "Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer." *Science* no. 324 (5933):1457-61. doi: 10.1126/science.1171362.

Oliver, M. H., N. K. Harrison, J. E. Bishop, P. J. Cole, and G. J. Laurent. 1989. "A rapid and convenient assay for counting cells cultured in microwell plates: application for assessment of growth factors." *J Cell Sci* no. 92 (Pt 3):513-8.

Orr, B., and D. A. Compton. 2013. "A double-edged sword: how oncogenes and tumor suppressor genes can contribute to chromosomal instability." *Front Oncol* no. 3:164. doi: 10.3389/fonc.2013.00164.

Orth, J. D., R. H. Kohler, F. Foijer, P. K. Sorger, R. Weissleder, and T. J. Mitchison. 2011. "Analysis of mitosis and antimitotic drug responses in tumors by in vivo microscopy and single-cell pharmacodynamics." *Cancer Res* no. 71 (13):4608-16. doi: 10.1158/0008-5472.can-11-0412.

Pearson, H.B., and N. Pouliot. 2000. Modeling Metastasis In Vivo.

Peters, J. M. 2006. "The anaphase promoting complex/cyclosome: a machine designed to destroy." *Nat Rev Mol Cell Biol* no. 7 (9):644-56. doi: 10.1038/nrm1988.

Prigent, C., and S. Dimitrov. 2003. "Phosphorylation of serine 10 in histone H3, what for?" *J Cell Sci* no. 116 (Pt 18):3677-85. doi: 10.1242/jcs.00735.

Puck, T. T., and P. I. Marcus. 1956. "Action of x-rays on mammalian cells." *J Exp Med* no. 103 (5):653-66.

Roschke, A. V., G. Tonon, K. S. Gehlhaus, N. McTyre, K. J. Bussey, S. Lababidi, D. A. Scudiero, J. N. Weinstein, and I. R. Kirsch. 2003. "Karyotypic complexity of the NCI-60 drug-screening panel." *Cancer Res* no. 63 (24):8634-47.

Rubin, E. H., and D. G. Gilliland. 2012. "Drug development and clinical trials--the path to an approved cancer drug." *Nat Rev Clin Oncol* no. 9 (4):215-22. doi: 10.1038/nrclinonc.2012.22.

Sarasin, A. 2003. "An overview of the mechanisms of mutagenesis and carcinogenesis." *Mutat Res* no. 544 (2-3):99-106.

Sausville, E. A. 2004. "Aurora kinases dawn as cancer drug targets." *Nat Med* no. 10 (3):234-5. doi: 10.1038/nm0304-234.

Schmidt, M., Y. Budirahardja, R. Klompmaker, and R. H. Medema. 2005. "Ablation of the spindle assembly checkpoint by a compound targeting Mps1." *EMBO Rep* no. 6 (9):866-72. doi: 10.1038/sj.embor.7400483.

Schmidt, M., and R. H. Medema. 2006. "Exploiting the compromised spindle assembly checkpoint function of tumor cells: dawn on the horizon?" *Cell Cycle* no. 5 (2):159-63.

Siegel, R., D. Naishadham, and A. Jemal. 2012. "Cancer statistics, 2012." *CA Cancer J Clin* no. 62 (1):10-29. doi: 10.3322/caac.20138.

Sihn, C. R., E. J. Suh, K. H. Lee, T. Y. Kim, and S. H. Kim. 2003. "p55CDC/hCDC20 mutant induces mitotic catastrophe by inhibiting the MAD2-dependent spindle checkpoint activity in tumor cells." *Cancer Lett* no. 201 (2):203-10.

Sirivatanauksorn, V., Y. Sirivatanauksorn, P. A. Gorman, J. M. Davidson, D. Sheer, P. S. Moore, A. Scarpa, P. A. Edwards, and N. R. Lemoine. 2001. "Non-random chromosomal rearrangements in pancreatic cancer cell lines identified by spectral karyotyping." *Int J Cancer* no. 91 (3):350-8.

Slee, R. B., B. R. Grimes, R. Bansal, J. Gore, C. Blackburn, L. Brown, R. Gasaway, J. Jeong, J. Victorino, K. L. March, R. Colombo, B. S. Herbert, and M. Korc. 2014. "Selective Inhibition of Pancreatic Ductal Adenocarcinoma Cell Growth by the Mitotic MPS1 Kinase Inhibitor NMS-P715." *Mol Cancer Ther* no. 13 (2):307-15. doi: 10.1158/1535-7163.mct-13-0324.

Smid, M., M. Hoes, A. M. Sieuwerts, S. Sleijfer, Y. Zhang, Y. Wang, J. A. Foekens, and J. W. Martens. 2011. "Patterns and incidence of chromosomal instability and their prognostic relevance in breast cancer subtypes." *Breast Cancer Res Treat* no. 128 (1):23-30. doi: 10.1007/s10549-010-1026-5.

Sofuni, A., H. Iijima, F. Moriyasu, D. Nakayama, M. Shimizu, K. Nakamura, F. Itokawa, and T. Itoi. 2005. "Differential diagnosis of pancreatic tumors using ultrasound contrast imaging." *J Gastroenterol* no. 40 (5):518-25. doi: 10.1007/s00535-005-1578-z.

Solimini, N. L., J. Luo, and S. J. Elledge. 2007. "Non-oncogene addiction and the stress phenotype of cancer cells." *Cell* no. 130 (6):986-8. doi: 10.1016/j.cell.2007.09.007.

Speicher, M. R., and N. P. Carter. 2005. "The new cytogenetics: blurring the boundaries with molecular biology." *Nat Rev Genet* no. 6 (10):782-92. doi: 10.1038/nrg1692.

Stucke, V. M., H. H. Sillje, L. Arnaud, and E. A. Nigg. 2002. "Human Mps1 kinase is required for the spindle assembly checkpoint but not for centrosome duplication." *EMBO J* no. 21 (7):1723-32. doi: 10.1093/emboj/21.7.1723.

Sullivan, K. M., and P. S. Kozuch. 2012. "Chemotherapy and other supportive modalities in the palliative setting for pancreatic cancer." *Cancer J* no. 18 (6):633-41. doi: 10.1097/PPO.0b013e318275896f.

Tang, Y. C., B. R. Williams, J. J. Siegel, and A. Amon. 2011. "Identification of aneuploidy-selective antiproliferation compounds." *Cell* no. 144 (4):499-512. doi: 10.1016/j.cell.2011.01.017.

Tardif, K. D., A. Rogers, J. Cassiano, B. L. Roth, D. M. Cimbora, R. McKinnon, A. Peterson, T. B. Douce, R. Robinson, I. Dorweiler, T. Davis, M. A. Hess, K. Ostanin, D. I. Papac, V. Baichwal, I. McAlexander, J. A. Willardsen, M. Saunders, H. Christophe, D. V. Kumar, D. A. Wettstein, R. O. Carlson, and B. L. Williams. 2011. "Characterization of the cellular and antitumor effects of MPI-0479605, a small-molecule inhibitor of the mitotic kinase Mps1." *Mol Cancer Ther* no. 10 (12):2267-75. doi: 10.1158/1535-7163.mct-11-0453.

Thompson, S. L., S. F. Bakhoum, and D. A. Compton. 2010. "Mechanisms of chromosomal instability." *Curr Biol* no. 20 (6):R285-95. doi: 10.1016/j.cub.2010.01.034.

Thompson, S. L., and D. A. Compton. 2008. "Examining the link between chromosomal instability and aneuploidy in human cells." *J Cell Biol* no. 180 (4):665-72. doi: 10.1083/jcb.200712029.

Tighe, A., O. Staples, and S. Taylor. 2008. "Mps1 kinase activity restrains anaphase during an unperturbed mitosis and targets Mad2 to kinetochores." *J Cell Biol* no. 181 (6):893-901. doi: 10.1083/jcb.200712028.

Torres, E. M., T. Sokolsky, C. M. Tucker, L. Y. Chan, M. Boselli, M. J. Dunham, and A. Amon. 2007. "Effects of aneuploidy on cellular physiology and cell division in haploid yeast." *Science* no. 317 (5840):916-24. doi: 10.1126/science.1142210.

Wang, Q., T. Liu, Y. Fang, S. Xie, X. Huang, R. Mahmood, G. Ramaswamy, K. M. Sakamoto, Z. Darzynkiewicz, M. Xu, and W. Dai. 2004. "BUBR1 deficiency results in abnormal megakaryopoiesis." *Blood* no. 103 (4):1278-85. doi: 10.1182/blood-2003-06-2158.

Weiss, E., and M. Winey. 1996. "The Saccharomyces cerevisiae spindle pole body duplication gene MPS1 is part of a mitotic checkpoint." *J Cell Biol* no. 132 (1-2):111-23.

Willard, H. F. 1985. "Chromosome-specific organization of human alpha satellite DNA." *Am J Hum Genet* no. 37 (3):524-32.

Williams, B. R., V. R. Prabhu, K. E. Hunter, C. M. Glazier, C. A. Whittaker, D. E. Housman, and A. Amon. 2008. "Aneuploidy affects proliferation and spontaneous immortalization in mammalian cells." *Science* no. 322 (5902):703-9. doi: 10.1126/science.1160058.

Yang, R., M. Niepel, T. K. Mitchison, and P. K. Sorger. 2010. "Dissecting variability in responses to cancer chemotherapy through systems pharmacology." *Clin Pharmacol Ther* no. 88 (1):34-8. doi: 10.1038/clpt.2010.96.

Yang, Z., J. Loncarek, A. Khodjakov, and C. L. Rieder. 2008. "Extra centrosomes and/or chromosomes prolong mitosis in human cells." *Nat Cell Biol* no. 10 (6):748-51. doi: 10.1038/ncb1738.

Yu, H. 2002. "Regulation of APC-Cdc20 by the spindle checkpoint." *Curr Opin Cell Biol* no. 14 (6):706-14.

Yu, H. 2007. "Cdc20: a WD40 activator for a cell cycle degradation machine." *Mol Cell* no. 27 (1):3-16. doi: 10.1016/j.molcel.2007.06.009.

Yuan, B., Y. Xu, J. H. Woo, Y. Wang, Y. K. Bae, D. S. Yoon, R. P. Wersto, E. Tully, K. Wilsbach, and E. Gabrielson. 2006. "Increased expression of mitotic checkpoint genes in breast cancer cells with chromosomal instability." *Clin Cancer Res* no. 12 (2):405-10. doi: 10.1158/1078-0432.ccr-05-0903.

Zhang, Xiao-Xiang. 2005. Chromosome Instability. In *The principles of clinical cytogenetics*. Totowa, N.J.: Humana Press.

CURRICULUM VITAE

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Education

- **MS, Medical and Molecular Genetics** **Graduated in 12/2014**
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Research experience

Graduate Student Research Assistant **9/2012 – 12/2013**
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- Investigated the effects of a small molecule chemotherapeutic drug, mitotic kinase MPS1 inhibitor, on normal human adipose stem cells in comparison with pancreatic cancer cells by performing long and short term proliferation assays as well as techniques such as Western Blot, Immunocytochemistry and Fluorescence *in situ* hybridization
- Assessed micronuclei formation in cultured human cells as an indirect measurement of chromosomal instability
- Maintained the growth of human and mice tumor and non-tumor cell lines such as pancreatic ductal adenocarcinoma cells, telomerase-immortalized pancreatic ductal epithelial cells, fibrosarcoma cells and adipose stem cells

Cytogenetic Laboratory Experience **1/2013 – 5/2013**
Cytogenetic Division,
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Performed techniques such as karyotyping and multicolor fluorescence in situ hybridization (M-FISH) analysis to detect and analyze chromosomal abnormalities in cytogenetic disorders

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Analyzed the effects of waste water from different industrial lines in promoting the errors in stages of mitosis in *Allium cepa* root tips and on anatomic malformations in the development of early chick embryos

Publications

- Kononenko AV, **Bansal R**, Grimes BR, Masumoto H, Earnshaw WC, Larionov V, Kouprina N. A portable BRCA1-HAC module for analysis of tumor suppressor function of BRCA. *Nucleic Acids Research*. First published online September 26, 2014 doi:10.1093/nar/gku870
- Grimes BR, Slee RB, **Bansal R**, Gore J, Blackburn C, Brown L, Gasaway R, Jeong J, March KL, Colombo R, Herbert BS, Korc M. Selective inhibition of pancreatic ductal adenocarcinoma cell growth by the mitotic MPS1 kinase inhibitor, NMS-P715. *Molecular Cancer Therapeutics.* February 2014 13(2); 307-15.
- Lee HS, Lee NC, Grimes BR, Samoshkin A, Kononenko AV, **Bansal R**, Masumoto H, Earnshaw WC, Kouprina N, Larionov V. A new assay for measuring chromosomal instability (CIN) and identification of drugs that elevate CIN in cancer cells. *BMC Cancer*. May 2013; 13:252

Poster presentations

- **Bansal R**, Blackburn C, Brown L, Gasaway R, Victorino J, Jeong J, Gore J, March KL , Herbert BS, Colombo R, Korc M, Slee RB and Grimes BR. An inhibitor of the mitotic kinase, MPS1, is selective towards pancreatic cancer cells. (2014). 5th Annual Midwest Graduate Research Symposium. March 29. University of Toledo, Toledo, Ohio, USA
- **Bansal R**, Blackburn C, Brown L, Gasaway R, Victorino J, Jeong J, Gore J, March KL , Herbert BS, Colombo R, Korc M, Slee RB and Grimes BR. An inhibitor of the mitotic kinase, MPS1, is selective towards pancreatic cancer cells. (2014) Women in STEM Research Poster Session sponsored by IUPUI Women in Science. March 6. Indiana University Purdue University at Indianapolis, Indianapolis, Indiana, USA
- **Bansal R**, Blackburn C, Brown L, Gasaway R, Victorino J, Jeong J, Gore J, March KL , Herbert BS, Colombo R, Korc M, Slee RB and Grimes BR. An inhibitor of the mitotic kinase, MPS1, is selective towards pancreatic cancer cells. (September 2013) Department of Medical and Molecular Genetics Poster Session. September 17. Indiana University School of Medicine, Indianapolis, Indiana, USA
- Victorino J, Slee RB, **Bansal R,** Korc M, Grimes BR. Targeting Chromosome Instability (CIN) in pancreatic cancer. (2013) Bridges to the Doctorate Program Summer Poster Session. July 25-26. Indiana University Purdue University at Indianapolis, Indianapolis, Indiana, USA

- Slee RB, Grimes BR, **Bansal R**, Gore J, Blackburn C, Brown L, Gasaway R, Jeong J, March KL, Colombo R, Herbert BS, Korc M. Selective inhibition of pancreatic ductal adenocarcinoma cell growth by the mitotic MPS1 kinase inhibitor, NMS-P715. (2013) Cancer Research Day. May 22. Indiana University School of Medicine, Indianapolis, Indiana, USA
- Blackburn C, Slee RB, **Bansal R**, Brown L, March KL, Herbert BS, Korc M, Grimes BR. The Mitotic Kinase, MPS1, as a Therapeutic Target in Pancreatic Cancer. (2013). Indiana University Life Health Sciences Internship Program Poster Session. April 12. Indiana University School of Medicine, Indianapolis, Indiana, USA.
- Brown L, Slee RB, **Bansal R**, Blackburn C, March KL, Herbert BS, Korc M, Grimes BR. (2013). Human Adipose Stem Cells are more Resistant than Pancreatic Cancer Cells to NMS-P715, a Mitotic MPS1 Kinase Inhibitor. Indiana University Life Health Sciences Internship Program Poster Session. April 12. Indiana University School of Medicine, Indianapolis, Indiana, USA

Awards

Poster Presentation Award **March 2014**
Third prize in Graduate Division
Women in STEM Research Poster Session, IUPUI, Indianapolis, IN

Campus and community involvement

- **Volunteer Laboratory Assistant for Human Tissue Collection Event** **1/2013 – 11/2014**
  Susan G. Komen for the Cure Tissue Bank,
  Indiana University Simon Cancer Center, Indianapolis, IN
- **International Graduate Welcome Volunteer** **7/2013 – 5/2014**
  Indiana University Purdue University, Indianapolis, IN
- **Chromosome Research Group** **3/3013 – 5/2014**
  Indiana University School of Medicine, Indianapolis, IN
- **Class Coordinator** **11/2008 – 1/2009**
  Fergusson College, Pune, India
  Represented the First Year Class of Bachelor of Science program in Biotechnology for the annual departmental festival, ‘Chimera’