Charles University

Faculty of Science

Study programme: Molecular and Cell Biology, Genetics and Virology

Mgr. Miroslav Přibyl

Mechanisms of phenotypic plasticity induced by genotoxic stress

Mechanismy fenotypové plasticity nádorových buněk indukované genotoxickým stresem

Doctoral thesis

Supervisor:

MUDr. Zdeněk Hodný, CSc.

Prague, 2020

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 25. 11. 2020

Mgr. Miroslav Přibyl

Podpis:

Acknowledgement

I would like to thank everyone who helped me to get to this point in my studies. I appreciate every help, every idea, and every struggle that got me here, where I may present the results of work that we, me and my colleagues, accomplished.

I would like to thank to Dr. Hodný, who guided me in my doctoral journey. To Dr. Hodný and prof. Bártek for allowing me to work in their department. To all members of the department for their support and relationships we created. I am very grateful for that.

Last but not least, I would like to thank to my family, who supported me on this journey.

And I would like to thank everyone who never doubted in me. Thank you.

Love you all.

Contents

Abbreviations

ADAM17	A disintegrin and metalloprotease domain 17
Akt	Thymomas of AKR (akv retrovirus) mice
Arg-1	Arginase 1
BCL-2	B-cell lymphoma 2
BCL-XL	B-cell lymphoma-extra large
BMP7	Bone morphogenetic protein 7
BORIS	Brother of the regulator of imprinted sites
CAR-T	Chimeric antigen receptor T cell
CCL2	C-C motif chemokine ligand 2
CD	Cluster of differentiation
CpGs	Cytosine phosphate guanine sites
CTLA-4	Cytotoxic T-lymphocyte antigen 4
CXCL10	C-X-C motif chemokine ligand 10
CXCL9	C-X-C motif ligand 9
DNA	Deoxyribonucleic acid
Erk	Extracellular signal-regulated kinase
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GSK3b	Glycogen synthase kinase 3 β
Gy	Gray (the absorption of one joule of radiation energy per kilogram of matter)
HIF-1A	Hypoxia-inducible factor 1-α
HLA	Human leukocyte antigen
HUVEC	Human umbilical vein endothelial cells
IDO-1	Indoleamine-pyrrole 2,3-dioxygenase
IFN	Interferon
IL	Interleukin
JAK1/2	Janus kinase 1/2
MAGE-A1	Melanoma-associated antigen 1
MCL1	Induced myeloid leukaemia cell differentiation protein
M-CSF	Macrophage colony-stimulating factor
MHC	Major histocompatibility complex
mRNA	messenger RNA

MYCK	Myosin light chain kinase
NY-ESO-1	New York esophageal squamous cell carcinoma-1
PCR	Polymerase chain reaction
PD-L1	Programmed death-ligand 1
POSTN	Periostin
RNA	Ribonucleic acid
SDS/PAGE	Sodium dodecylsulphate/polyacrylsmide gel electrophoresis
SNAI1	Snail family transcriptional repressor 1
SNAI2	Snail family transcriptional repressor 2
SOX2	SRY (sex determining region Y)-box 2
STAT1	Signal transducer and activator of transcription 1
TGFβ	Transforming growth factor β
Th2	T helper 2 cells
TNFα	Tumour necrosis factor α
TP53	Transformation-Related Protein 53
TRAF6	TNF receptor associated factor 6
tRNA	Transfer RNA
TWIST1	Twist-related protein 1
TWIST2	Twist-related protein 2
VCAM1	Vascular cell adhesion protein 1
VEGF	Vascular endothelial growth factor
Wnt	Wingless-type
ZEB1	Zinc finger e-box binding homeobox 1
ZEB2	Zinc finger e-box binding homeobox 2

1. Abstract

Therapy resistance of malignant cells represents the main reason responsible for the failure of cancer therapy. The growth of malignant cells at primary tumour sites but most importantly the dissemination of tumour cells and their growth at secondary sites, are the main reasons why patients eventually succumb to the disease. Even novel immune-based therapies find their limitation in most tumour types. The therapy resistance is mediated by the tumour cells but also by other cellular components of the tumour microenvironment. Understanding the tumour cells mechanisms and the tumour microenvironment features responsible for therapy resistance enables the development of novel therapeutic strategies.

Here, we show that ionizing irradiation, 5-azacytidine, and IFNγ treatments induced expression of *suprabasin* (*SBSN*) and therapy-resistant low-adherent phenotype in cancer cells. Knockdown of *SBSN* resulted in suppression of the phenotype. Next, we identified aberrantly elevated *SBSN* in the bone marrow of a subgroup of myelodysplastic syndromes (MDS) patients. *SBSN* was expressed by myeloid-derived suppressor cells (MDSCs) and showed significant anti-correlation with T cell abundance and CCL2 levels, hence promises a prognostic value in clinical use. We compiled the most of the relevant knowledge of *SBSN* together with our results. Data presented in this thesis point to an important role of *SBSN* oncogene in therapy resistance and its diagnostic and prognostic potential in MDS.

Abstrakt

Rezistence nádorových buněk vůči léčbě představuje hlavní důvod neúspěšné nádorové terapie. Růst nádorových buněk v primárním místě nádoru nebo jejich šíření a růst v sekundárních oblastech jsou hlavní důvody, proč pacienti nemoci podlehnou. I moderní terapie založené na aktivaci imunitního systému nachází své hranice u mnohých typů nádorů. Rezistence vůči léčbě je zprostředkována nejen nádorovými buňkami, ale i jinými komponenty nádorového mikroprostředí. Porozumění mechanizmům nádorové rezistence zprostředkované nádorovými buňkami, ale i nádorovému mikroprostředí umožní vývoj nových terapeutických přístupů.

V této práci představujeme naše nedávné výsledky. Ozařování, 5-azacytidin i IFNγ indukují expresi genu *suprabasin* (*SBSN*) a vznik fenotypu nádorových buněk, který je zodpovědný za resistenci vůči terapii a projevuje se nízkou adhezivitou a kmenovými

vlastnostmi nádorových buněk. Snížení exprese genu *SBSN* vedlo k potlačení tohoto fenotypu. Rovněž jsme identifikovali aberantně zvýšenou expresi genu *SBSN* v kostní dřeni podskupiny pacientů trpících myelodysplastickými syndromy (MDS). Exprese *SBSN* byla zprostředkována supresorovými buňkami odvozenými z myeloidní řady (MDSCs) a hladina SBSN negativně korelovala se zastoupením T buněk a koncentrací CCL2 v kostní dřeni pacientů. Z toho vychází možný prognostický potenciál SBSN u MDS. V této práci shrnujeme nejdůležitější poznatky o *SBSN* spolu s našimi výsledky. Data zde prezentovaná ukazují na významnou roli onkogenu *SBSN* v nádorové rezistenci a jeho potenciál jako diagnostického a prognostického biomarkeru u MDS.

2. Introduction

2.1 Cancer

Cancer is the second leading cause of death worldwide (Roth et al., 2018). Cancer is characterized as an aberrant growth of cells resulting in damage of surrounding tissue or dysfunction of an organ resulting in death of an individual.

The current understanding of cancer seems to be relatively vast. Certain parts of carcinogenesis in adults are well described, whereas others are lacking. For example, novel mechanisms of oncogenic signalling, cancer resistance or tumour cells spreading are being described almost daily. Yet we do not have answers for the most important questions: why cancer starts and can it be ended?

Certainly, environmental effects and lifestyle factors, such as unhealthy diet, smoking, or alcohol are associated with carcinogenesis, but we can ask why do individuals use these damaging substances? Oncoviruses promote tumorigenesis, hence are responsible for several tumour types (Mui et al., 2017) and, in some cases, for subsequent spreading (Bishop et al., 2012; Perri et al., 2018). Besides these, the origin of most cancer types (not the most prevalent cancer types) is considered as 'bad luck'. A random mutation promotes dysregulated growth of cells and accumulation of other mutations, thus resulting in a tumour mass disrupting the function of tissues. Eventually, tumour cells' spreading promotes growth at secondary sites, growing into overt metastases destroying affected organs. But the somatic mutation theory has its weak spots.

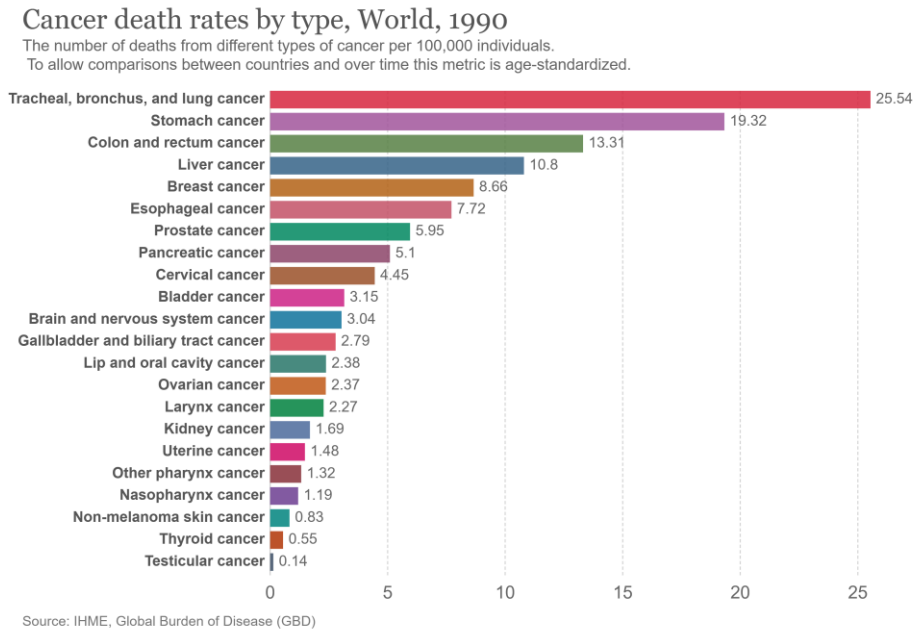
A single mutation resulting in dysregulated growth would have to overcome the function of complementary allele, senescence, and immune surveillance. Therefore, mutations are rather a feature of cancer, a symptom of the disease. Cells adapt to changing environment without increasing the mutational burden (Pisco et al., 2013). This may result in dysregulated growth, which is subsequently responsible for increase in the mutational burden. Chronic inflammation promotes DNA damage, if unrepaired the accumulation of mutations is inevitable (Coussens and Werb, 2002). Immune surveillance, the essential feature of immune system, mediates clearance of malignant cells before the tumour mass is established (Swann and Smyth, 2007). However, if the immune system is suppressed via chronic inflammation, diet, drugs, emotional imbalance and stress, or separation (Friedman et al., 2003; Greten and Grivennikov, 2019; Kiecolt-Glaser and Glaser, 1995;

O'Leary, 1990; Seitz and Stickel, 2007), then immune surveillance may be disrupted. Therefore, understanding the complex nature of cancer is essential.

2.2 Cancer death rates

In 2019 ~10 million people died worldwide from cancer, it was 5.6 million in 1990, and is predicted to be ~13 million in 2030 (Ferlay et al., 2019; Roser and Ritchie, 2015). Therefore, age of cancer is yet to come, but cancer therapy faces the same challenges as ever before.

A parameter of cancer prognosis, the overall 5-year survival, has increased from 50.3 to 67% in the last 50 years, and cancer death rates (age-standardized cancer-caused deaths per 100.000 people in population) has declined by 15% over the last 30 years worldwide (Roser and Ritchie, 2015). It seems that cancer therapy has become more efficient, but this is not a correct interpretation. Both metrics are affected by several biases responsible for this seemingly positive progress. Besides others, the overall 5-year survival is affected by 'lead time bias' and 'overdiagnosis bias' (Gigerenzer and Wegwarth, 2013). Better diagnostic tools allow earlier diagnosis or diagnosis of asymptomatic and possibly never-to-be symptomatic diseases. This results in the overall 5-year survival increase, yet patients die at similar age. In general, counts of cancer-caused deaths are affected by growing and aging population, therefore normalization to rates and age-standardization are required, hence the term 'age-standardized cancer death rates'. In last 30 years, colon and stomach cancer showed decrease in cancer death rates (from 19.3 to 10.9 per 100.000 in case of stomach cancer and from 13.3 to 11.5 per 100.000 in case of colon cancer), but other 20 types of most prevalent cancer types has barely changed (these include lung, ovarian, brain, prostate and cervical cancer, etc.; Figure 1 and 2). Therefore, cancer death rates decrease in colon and stomach cancer is responsible for documented overall decrease in the metric. Better and earlier diagnosis is essential for decrease in cancer death rates in these two cancer types, which is certain in case of colon cancer (Roser and Ritchie, 2015). Still, surgical approach is the first-line option in both cases. Therefore cancer therapy has not shown any significant effect in last 30 years. Question remains, *why is that*?



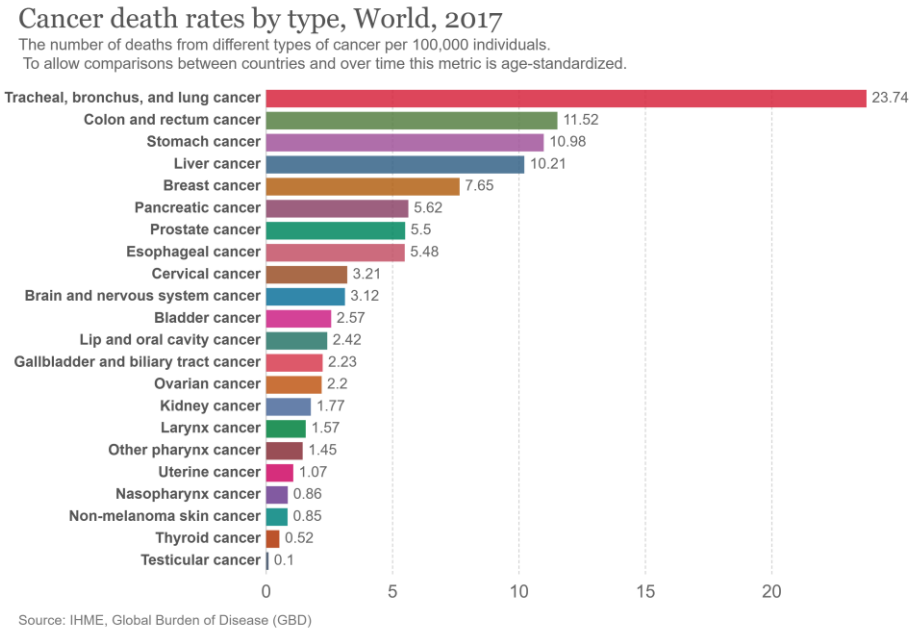


Figure 1. **Worldwide cancer death rates in 1990 and 2017.** Cancer death rates (age-normalized cancer caused deaths per 100.000 people) of twenty deadliest cancers worldwide in 1990 and 2017. Adapted from (Roser and Ritchie, 2015).

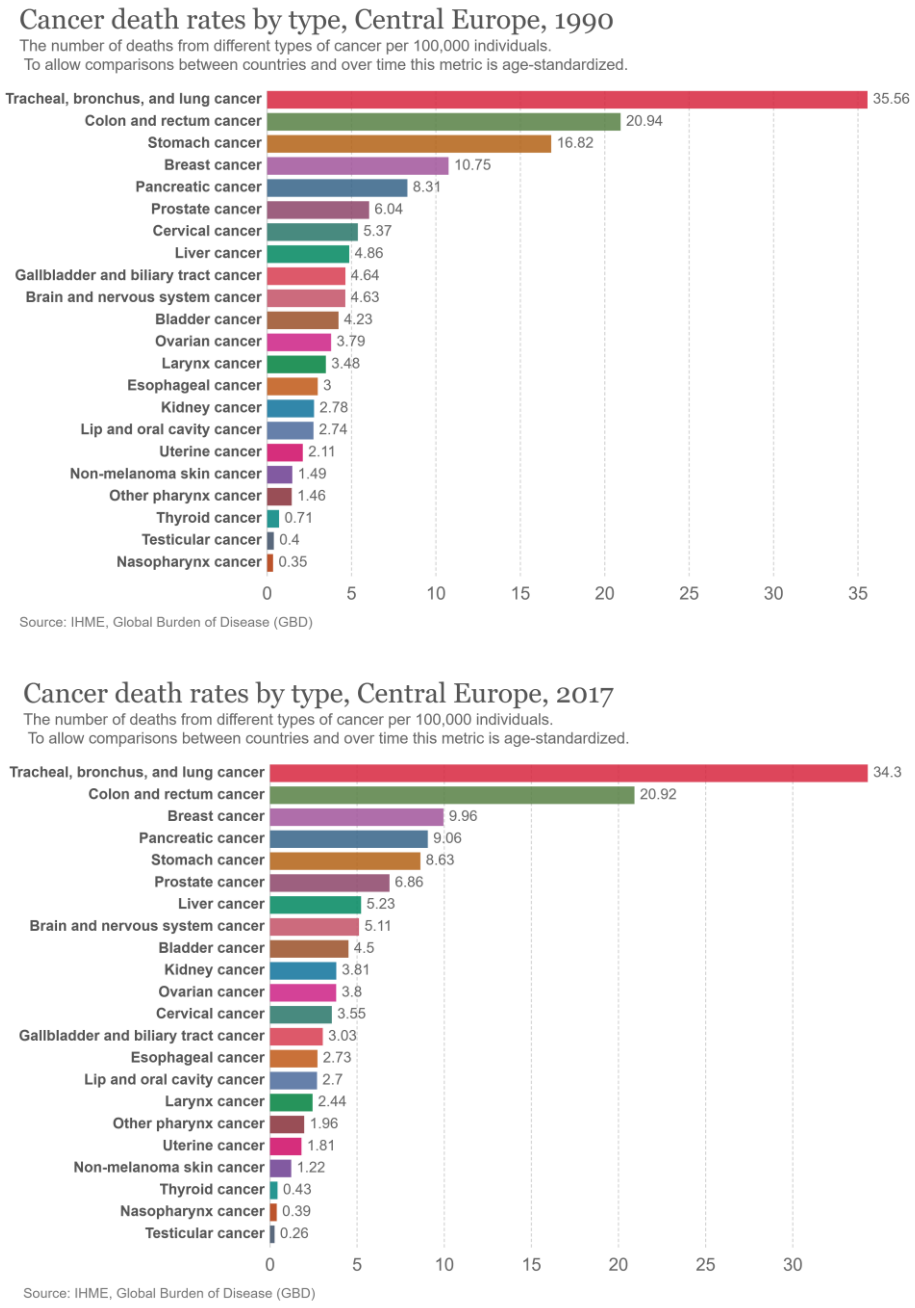


Figure 2. **Central Europe cancer death rates in 1990 and 2017.** Cancer death rates (age-normalized cancer caused deaths per 100.000 people) of twenty deadliest cancers in Central Europe in 1990 and 2017. Adapted from (Roser and Ritchie, 2015).

2.3 Cancer therapies

Following the surgical dissection, radiotherapy and chemotherapy remain the selected treatments for most tumours or unresectable types (Gilbert et al., 2013; Ledermann, 2018; Stupp et al., 2005; Tu and Hsu, 2018). For decades, chemotherapy agents, such as

methotrexate (Koźmiński et al., 2020), 5-azacytidine (Santini, 2019), or cisplatin (Dasari and Bernard Tchounwou, 2014) are still widely used. Certainly, novel approaches including immune-based therapies could not impact cancer death rates markedly just yet. Immune checkpoint inhibitors (ICI) have shown their use in melanoma (Herrscher and Robert, 2020; Zimmer et al., 2020). On the contrary, ICI as monotherapies did not provide satisfactory results in other cancer types (Huang et al., 2020) and resistances towards immunotherapies are already documented (Shah and Fry, 2019; Wylie et al., 2019). Thus, past failures represent future struggles.

2.3.1 Radiotherapy

The selection of therapy is based upon the stage of cancer and other criteria such as patients' age. Patients with unresectable tumours are usually treated with radiotherapy or its combination with chemotherapy (Durante and Loeffler, 2010). Unresectable tumours are represented with, for instance, prostate cancer (treatment together with androgen deprivation therapy (Dal Pra et al., 2010)), unresectable bladder cancer, unresectable non-small cell lung cancer (NSCLC) and many others (The Royal College of Radiologists, 2019).

Different radiotherapy setups and regimes are used. However, most commonly applied setups utilize 1.8 – 2 Gy per session over the course of 2 – 7 weeks. Therefore, the total dosage range is 20 – 70 Gy (Durante and Loeffler, 2010; The Royal College of Radiologists, 2019).

Ionizing irradiation induces DNA damage that requires extensive repairs. This is promoted directly via irradiation, or via irradiation-induced free radicals (mostly reactive oxygen species; ROS) which also promote DNA damage. Most of the cells of tumour mass are considered to be highly proliferating due to high expression of oncogene(s). Therefore, quickly cycling cells are struggling with the repair. In mitosis, pro-apoptotic signals are promoted by damaged chromosomes and the cells eventually undergo cell death (Eriksson and Stigbrand, 2010). Extensive DNA damage may also promote p53-dependent apoptosis in interphase (Fridman and Lowe, 2003).

Besides cancer cell death, radiotherapy also promotes senescence. Senescence is considered as primary tumourigenesis barrier as it certainly stops affected tumour cells from growth, however senescent cells are also associated with cancer resistance, growth,

and spreading. Hence induction of senescence with radiotherapy may represent a desired, but also unwanted effect (Tabasso et al., 2019). Importantly, other therapeutic approaches, such as genotoxic chemotherapies, also induced senescence in affected tumour cells (Venturelli et al., 2013). Therefore, targeting of senescent cells with senolytics, the senescent cell eliminating drugs, is proposed to be combined with cancer therapy (Demaria et al., 2017; Short et al., 2019).

Therefore, irradiation-induced DNA damage results in cell death or senescence, thus suppressing the growth of malignant cells. This effect is also induced with many chemotherapeutic agents (Demaria et al., 2017; Short et al., 2019).

2.3.2 Chemotherapy

Chemotherapy includes large variety of small molecules which affect most of the cells in patients' body. Various chemotherapeutic agents promote different effects, but eventually they all promote cell death via apoptotic pathway. Hence, apoptosis-avoiding mechanisms are often upregulated in tumour cells. Additionally, other mechanisms, such as export of the agents or their modification into inactive compounds are also utilized by tumour cells (Housman et al., 2014).

2.3.2.1 5-azacytidine and 5-aza-2'-deoxycytidine

5-azacytidine (5-AC; Vidaza®) (Šorm et al., 1964) and 5-aza-2'-deoxycytidine (5-dAC; Decitabine) (Pliml and Šorm, 1964) (Figure 3) were originally synthetized in Czechoslovakia in 1964. 5-AC represents an analogue of cytidine, whereas 5-dAC is an analogue of deoxycytidine. Both are hypomethylating agents, hence they promote reduction in methylation of DNA. Since DNA methylation is associated with silencing of genes, treatment with 5-AC or 5-dAC promotes changes in expression profiles of affected cells, but also other effects (Christman, 2002).

5-AC incorporates into both, DNA and RNA. Incorporation into mRNA or tRNA affects translation. 5-AC promotes multiple effects, and this is likely dependent upon its concentration used, cellular uptake, efflux, and mechanisms responsible for its conversion. 5-dAC incorporates into DNA only and this is likely responsible for its lower cytotoxicity. This also explains its higher efficiency in lower concentration (Christman, 2002). Therefore, the effect of 5-AC and 5-dAC in DNA is the same. Once incorporated into

DNA, both molecules may result in chromosome breakage. DNA methyltransferase 1 (DNMT1) operates on cytosine residues in DNA, however, 5-AC or 5-dAC residues are also recognized. In an attempt to promote methylation of 5-AC or 5-dAC, DNMT1 becomes irreversibly bound onto DNA. This results to loss of methylation, including CpGs islands of promoter sites, resulting in dysregulated expression of thousands of genes. In this manner, the manifestation of 5-AC or 5-dAC effect is dependent upon DNMT1 abundance in a particular cell (Christman, 2002).

Once DNMT1 reacts with 5-dAC incorporated in DNA, the DNMT1:5-dAC adduct is created. The adducts are ultimately responsible for the 5-dAC-induced cytotoxicity (Juttermann et al., 1994). These adducts promoted p53-mediated DNA damage response in colon tumour cells (Karpf et al., 2001), which is partially responsible for the 5-dAC-mediated effect. Additionally, 5-dAC showed effect *in vitro* targeting human-derived tumour cells, when these cells showed prolonged cell cycle compared to treated fibroblasts (Bender et al., 1998). Similar results were achieved *in vivo*, when using a mice leukemic model (Covey and Zaharko, 1985).

The repair of the DNMT1:5-AC adducts leads to degradation of DNMT1 and its interacting components of epigenome, including histones with repressive marks (Shen and Laird, 2013), this eventually enables the expression of downstream genes.

Tumour cells show altered DNA methylation. It is recognized, that overall DNA methylation is reduced in tumour cells, but some genes are selectively hypermethylated, hence suppressed in expression (Costello et al., 2000). These include tumour suppressor genes (Sakai et al., 1991; Santini et al., 2001). Reactivation of tumour suppressor genes in affected cells may result in inhibition of cell cycle or induction of cell death.

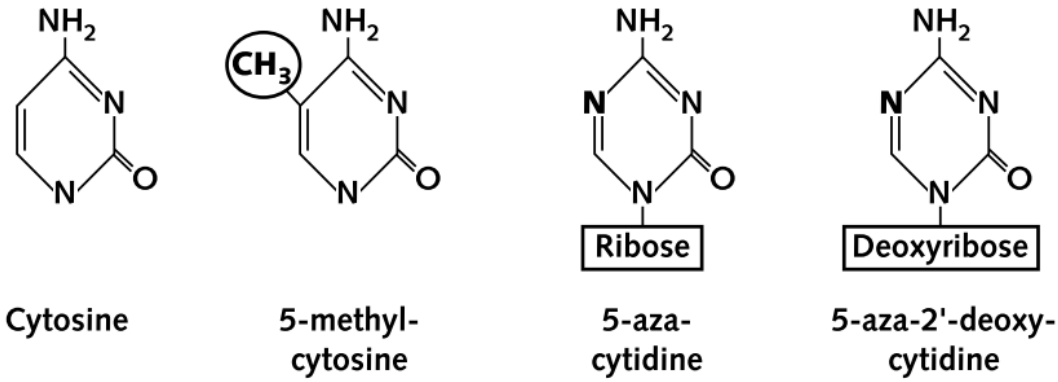


Figure 3. **Comparison of schemes of cytosine, 5-methyl-cystine, 5-azacytidine, and 5-aza-2´-deoxycytidine.** Both, cytosine and 5-methyl-cytosine represent diazines molecules, whereas 5-azacytidine and 5-aza-2´-deoxycytidine are triazines. As the names suggest, 5-azacytidine and 5-aza-2´-deoxycytidine contain nitrogen instead of carbon at position 5 of pyrimidine ring. Adapted from (Santini et al., 2001).

Altogether, both 5-AC and 5-dAC mediate various effects. Which effects are induced depends on concentrations of the agent, or resistance mechanisms of the cells. However, the treatment may also lead to dysregulated expression of multiple genes, and their effect is hard to prognosticate. Importantly, the effect of 5-AC and 5-dAC in healthy fibroblasts is limited (Bender et al., 1998), hence providing a certain level of specificity towards malignant cells.

2.3.3 Immunotherapy

Immunotherapy brought new options with great potential into cancer treatment. The approach is predominantly based upon stimulation of immune response of the host through various treatments or techniques. These include adoptive T cell transfer (Humphries, 2013), vaccination via dendritic cells (Van Willigen et al., 2018), ICI (Pardoll, 2012), and several others. CAR-T cells recognizing specific antigens may also be included to this group (June et al., 2018). Similarly, the inhibitory antibodies may also be considered as an immunotherapy technique but their general mechanism of action differs.

To promote efficient immune response which results in tumour cells elimination, antigen presentation by the tumour cells is crucial. Altered peptides of mutated genes (neoantigens) could be presented via antigen presentation mechanisms. Therefore, induction of neoantigen expression, or enhancement of this process, would promote

desired immune response (Wang and Cao, 2020). Additionally, expression of tissue-restricted genes, such as cancer testis antigens (CTAs), towards which the central tolerance is not established (Suri et al., 2012), would also promote immune response. In order to achieve this effect, several agents promoting epigenetic changes were suggested as a co-treatment with immune-based therapies. These include hypomethylating agents, such as 5-AC or 5-dAC.

Indeed, both 5-AC and 5-dAC induces expression of tissue-restricted genes, including CTAs (James et al., 2006; Karpf et al., 2004; Suri et al., 2012) e.g. NY-ESO-1, MAGE-A1, and master regulator of CTAs expression, BORIS (Karpf, 2006). Additionally, expression of silenced neoantigens could be elevated following 5-AC treatment; hence these antigens could be recognized with T cells as well (Wang and Cao, 2020). This would significantly help patients suffering from cancers that are associated with high mutational burden, such as melanomas (Chiappinelli et al., 2016). This has already been confirmed in mouse melanoma model (Chiappinelli et al., 2015), murine ovarian cancer models (Wang et al., 2015a) and murine pancreatic carcinoma models (Ebelt et al., 2020). Therefore, combination of immunotherapy with 5-AC was proposed.

Immunotherapy holds a great promise, especially when combined with corroborating treatments which elevate immune response enhancing stimuli.

2.4 Genotoxic stress

Mutational burden is certainly a hallmark of cancer (Hanahan and Weinberg, 2011). Genotoxic stress may promote changes in genomic DNA sequence. Unresolved or wrongly repaired damage in DNA results in aberration. These mutations are propagated into daughter cells, thus altered genes are expressed. This results in aberrant phenotype.

Multiple mechanisms are responsible for genotoxicity. These are external, such as ionizing radiation or chemical agents (alkylating agents or intercalations agents), or intrinsic, such as reactive oxygen species (ROS) generated from cellular respiration or errors of replication (Coates et al., 2005). Inflammation also promotes DNA damage and potentiates the increase in mutational burden (Coussens and Werb, 2002; Hubackova et al., 2015).

Highly proliferative cells, such as some cancerous cells, are indeed more sensitive to genotoxic stress. Additionally, mutations of DNA repair machinery genes (Fink et al., 1997) or their active silencing (Chen et al., 2010; Howard et al., 2009) are observed in cancer. This increases predispositions to other mutations to occur and leading eventually to cancer cell death. Hence genotoxic therapies provide reasonable solution.

Unfortunately, the mechanisms of resistance against radio- and chemo-therapy are being described. These include hypoxia of the tumour mass which provides radiotherapy resistance via reduced generation of double-strand breaks (Begg and Tavassoli, 2020). Hypoxia also promotes inflammation which enables immune evasion and spreading (Cummins et al., 2016). Similarly, multiple mechanisms enable chemotherapy resistance.

2.5 Cancer resistance

Cancer cells resistance remains the number one cause responsible for the therapy failure (Holohan et al., 2013). Secondly, tumour cells dissemination and subsequent growth at secondary sites are the predominant reasons why patients succumb to the disease (Klein, 2011).

Surgical resection of tumour mass does not guarantee complete elimination of every tumour cell, and in some cancer types or stages of progression, surgical resection is not an option; hence chemotherapy or other treatments are often applied. Despite the variety of therapies, all treatments must promote cell death of tumour cells. Therefore, cell death-evading mechanisms are upregulated in almost every cancer type. These include upregulation of anti-apoptotic genes (such as BCL-2 family of genes: *BCL2*, *BCL-X_L*, or *MCL1* (Kale et al., 2018)) and activation of pro-survival signalling pathways (e.g Akt or Erk pathways (Lu et al., 2020; Revathidevi and Munirajan, 2019)). Therefore, inhibiting these targets is of interest. Indeed, antagonists recognizing BCL-2 family proteins, such as Venetoclax, ABT-737, or S63845 are finding its way into clinics (Roberts et al., 2019; Yasuda et al., 2020). However, resistance toward these agents is already being described (Konopleva et al., 2006; Tausch et al., 2019). Similar is the case of inhibitors targeting pro-surviving pathways as potential therapeutic agents (Jaiswal et al., 2018).

Modify, and thus inhibiting chemotherapy agents, or their active efflux is also commonly utilized by tumour cells (Housman et al., 2014). ATP-binding cassette (ABC) genes represent transmembrane proteins encoding family of genes responsible for

chemotherapy resistance utilizing the drug efflux. *Multi-drug resistance protein 1* (*MDR1*) is most well studied gene of the family, yet other 48 members are identified. It is proposed that upon inhibition different members of the family may take on the actions of the inhibited member (Szakács et al., 2004). Additionally, vincristine induced *MDR1* expression in a leukemic models via Wnt pathway activation (Pisco et al., 2013), hence adaptive non-mutational mechanisms participate in cancer resistance. Altogether, this likely explains failures of ABC inhibitors in clinical trials (Pusztai et al., 2005; Ruff et al., 2009).

Upregulation of immune-evading mechanisms is also observed (Holohan et al., 2013; Kalbasi and Ribas, 2020; Schoenfeld and Hellmann, 2020). This is responsible for evading the immune surveillance prior therapy, but also responsible for immunotherapy resistances. Overexpression of IFNγ receptors (Pan et al., 2018) and activation of IFNγ response in tumour cells promotes resistance via upregulation of *CD274* (also known as programmed death-ligand 1; PD-L1) (Bellucci et al., 2015; Garcia-Diaz et al., 2017) or secretion of PD-L1-exposing exosomes (Chen et al., 2018). These mechanisms are partially responsible for observed resistance towards infiltrated T cells (Patel et al., 2017). Conversely, IFNγ also induces expression of genes encoding MHC I components (Basham and Merigan, 1983; Kalbasi and Ribas, 2020).

Antigen presentation via MHC I is essential for T cell-induced cell death of tumour cells. However, mechanisms responsible for downregulation MHC I are described. Some tumour types showed silencing of MHC I genes (Restifo et al., 1996; Sucker et al., 2014). The mediators of IFNγ signalling, *JAK1* and *JAK2* were documented to be inactivated by mutation in melanoma patients (Zaretsky et al., 2016), and *STAT1* was shown to be suppressed in mouse model (Benci et al., 2016). Additionally, hyperactivation of Erk pathway (Frederick et al., 2013), which is often mediated by activating mutation of the components of the pathway, was also shown to repress MHC I expression. All marking evolution of mutant clones.

Similar to radiotherapy or chemotherapy, IFNγ signalling also induces DNA damage and cellular senescence. This is mediated via ROS generation and induction of TGFβ (Braumüller et al., 2013; Hubackova et al., 2015). Hence immune editing may also induce genotoxic stress, which results in inhibition of proliferation.

Besides suppression of antigen presentation, Erk pathway induced expression of *IL6*, *IL10*, and *VEGF* paralogues (Sumimoto et al., 2006). These signalling molecules represent essential stimuli promoting immunosuppression and stimulation of dissemination. Next, Wnt pathway promoted *IL10* expression in melanoma as well (Yaguchi et al., 2012), and activation of Wnt in dendritic cells dampened their anti-tumorigenic function (Spranger et al., 2015). Furthermore, infiltration of T cells negatively correlated with Wnt pathway activation in colorectal cancer and ovarian cancer (Grasso et al., 2018; Jiménez-Sánchez et al., 2017). And β-catenin marked stemness of cancer cells in several tumour types (Malanchi et al., 2012; Vermeulen et al., 2010; Wang et al., 2015b). In general, cancer stem cells (CSCs) are therapy-resistant cells with tumour growth-initiating potential responsible for secondary site metastases.

Altogether, combination of therapeutic approaches including inhibitors of Erk or Wnt pathway together with immunotherapy are currently ongoing or reported (Ascierto et al., 2019; Ebert et al., 2016; Ribas et al., 2019) and showing promising response rates. However, tumour cells are not the only compartment responsible for immune suppression. Novel immune-based therapy approaches must face additional complications, which are promoted via suppressive cells of the tumour microenvironment (TME) (e.g. anti-inflammatory macrophages (also known as M2 macrophages) or myeloid-derived suppressor cells, MDSCs) (Nakamura and Smyth, 2020).

2.6 Tumour microenvironment

Besides tumour cells, the tumour mass is composed of various cells types. These include the cells of the tissue affected by malignant conditions, cancer-associated fibroblasts, endothelial cells, and senescent cells, but also cells of the immune system. Later group is consisted of anti-tumour and pro-tumour cells. Anti-tumour cells are represented by cytotoxic T lymphocytes, anti-tumour macrophages, dendritic cells, and natural killer (NK) cells. Pro-tumour cells are represented by anti-inflammatory macrophages, regulatory T cells (Tregs), and MDSCs. All these components create TME. In some cases, extracellular matrix (ECM) and nerves are also considered to be components of TME (Gabrilovich et al., 2012; Vitale et al., 2019; Whiteside, 2008; Zahalka and Frenette, 2020).

TME mediates a complex network of interaction, metabolites transfer, and signals, altogether enabling tumour growth, evolution, resistance, and spreading (Greten and Grivennikov, 2019). Inflammatory signalling represent a dominant feature of TME associated with its aforementioned properties. These include innate immune signals, such as alarmins, damage-associated molecular patterns (DAMPs), and also pathogen-associated molecular patterns (PAMPs). But also inflammatory cytokines of varying nature, such as IL-1β, IL-6, IL-10, TGFβ, TNFα and many others (Medzhitov, 2008). Despite certain effort, in most cases, the initial source of inflammation is not known. Infection, autoimmune response, or systemic chronic inflammations induced by other malignancies are often on the onset of tumourigenesis and related inflammation (Grivennikov et al., 2010). Note, therapy may also potentiate inflammatory conditions (Greten and Grivennikov, 2019). And inflammatory milieu promotes epigenetic changes similar to changes induced with activity of oncogenes (Schwitalla et al., 2013), hence accumulation of mutations is enabled.

Pro-inflammatory signals derived from TME stimulate anti-inflammatory response; however, the immune system is only partially responsible for the expression of pro-inflammatory stimuli (Greten and Grivennikov, 2019). The other components of TME, such as tumour cells, cancer-associated fibroblasts, and senescent cells are also responsible for secretion of pro-inflammatory molecules. Inflammatory signalling enhances stemness of the tumour mass (Francart et al., 2018), and its therapy resistance. These cells, however, are not the targets of anti-inflammatory response mediated by anti-inflammatory macrophages, MDSCs, and other cell types, which efficiently suppress cytotoxic cells with anti-tumour activities; hence the pro-tumour milieu is established (Gabrilovich et al., 2012).

The malignant growth of the tumour mass is also associated with reduced oxygen availability; hence multiple signals stimulate neovascularization of TME. HIF-1A stabilization is also associated with cancer stemness, and cancer stem cells possess tumour growth-reinitiating potential at primary or secondary sites (Chen et al., 2020). Formation of novel blood vessels allows tumour growth and dissemination.

Despite being commonly described as a feature of solid tumours, haematological diseases, such as leukaemia and lymphomas also possess TME (Höpken and Rehm, 2019). Certainly, aspects of these TME deviate from TME of solid tumours, but the cellular composition is similar. In recent years, MDSCs in the bone marrow of myelodysplastic

syndromes (MDS) patients, the pre-leukemic diseases, were proposed to promote immune evasion, but were also shown to promote erythropenia and to establish inflammatory milieu in the bone marrow of MDS patients (Chen et al., 2013). Therefore, MDSCs are also responsible for the features of the disease itself.

2.7 Tumour dissemination

Dissemination of tumour cells from the primary sites may result in growth at secondary sites, usually distant organs with certain degree of specificity for some cancer types (e.g. prostate carcinoma and bone metastases, sarcomas and lung metastases), but mostly affects sites with smaller capillaries, such as liver, brain, lungs, and bone marrow (Carbonell et al., 2009; Szmulewitz et al., 2015). The process of dissemination may occur throughout the course of tumourigenesis (Brastianos et al., 2015; Hüsemann et al., 2008; Klein, 2011). Some reports suggest that therapy may facilitate this process as well (Greten and Grivennikov, 2019). Loss of adhesion to surrounding cells and matrix, migration of the cells, entering lymphatics or bloodstream, and subsequently leaving the bloodstream, are events necessary prior the growth of disseminated cancer cells. These processes require phenotypic changes in primary tumour cells (Dongre and Weinberg, 2019; Williams et al., 2019).

Cancer cells exploit multiple states which may ultimately result in cancer dissemination. The epithelial-mesenchymal transition (EMT) represents a range of phenotypes associated with invasiveness of tumour cells. Importantly, EMT is promoted via malignant milieu of TME, and some of the stimuli are also provided by tumour cells themselves (Giannoni et al., 2010; Jing et al., 2011; Radisky et al., 2005; Williams et al., 2019). This includes signalling molecules, such as TGFβ (Xu et al., 2009), WNTs (Teeuwssen and Fodde, 2019), IL-6 (Ebbing et al., 2019), and mitogenic factors (Kim et al., 2016), as well as cell-to-cell contact-induced signalling via NOTCHs (Natsuizaka et al., 2017).

The signals of TME then induce expression of EMT master regulators: *SNAI1*, *SNAI2*, *TWIST1*, *TWIST2*, *ZEB1*, and *ZEB2*. These transcription factors orchestrate epigenetic changes and expression of thousands of genes responsible for suppression of epithelial phenotype, active remodelling of the surrounding matrix, and promotion of mesenchymal phenotype. These changes elevate invasiveness of the cells. Once leaving the

original milieu of primary tumour site, the migrating cells may spread into the distant organs, the secondary sites. There, the stimuli responsible for EMT are lost, thus the reverse process, the mesenchymal-epithelial transition (MET) occurs. State of cellular dormancy follows, but upon its exit, the out-migrated cells re-enter the cell cycle and promote growth of micrometastases; cancer cell microcolonies at the distant sites potentially initiating cancer growth upon escaping dormancy (Dongre and Weinberg, 2019).

Besides EMT, several other types of invasive phenotype were described. Cancer cells were shown to migrate collectively (Cheung et al., 2016). In this case, only some of the cells showed mesenchymal phenotype, whereas other cells of the migrating cluster showed rather epithelial phenotype (Cheung and Ewald, 2016; Cheung et al., 2016). Other types of migratory phenotypes are dependent upon interaction with neuron cells, which serve as a path of migration (Magnon et al., 2013). Stress conditions, such as hypoxia, may induce another transition from mesenchymal phenotype into amoeboid, so called the mesenchymal-to-amoeboid transition (MAT) (Lämmermann and Sixt, 2009; Lehmann et al., 2017). It is proposed, that the invasiveness of cancer cells with the amoeboid phenotype is not dependent upon remodelling of extracellular matrix. But the precise nature of this phenomenon is yet to be determined *in vivo* (Lämmermann and Sixt, 2009; Lehmann et al., 2017; Sabeh et al., 2009).

Nevertheless, the migratory phenotypes are commonly associated with expression of stem cell markers, such as *SOX2* or *CD44* (Al-Hajj et al., 2003; Alonso et al., 2011; Giannoni et al., 2010; Prince et al., 2007; Thiery et al., 2009; Vlashi and Pajonk, 2015). Most of the disseminated cells are likely tumour-initiating CSCs, or the migrating cells enter the state of stemness, thus become CSCs or cancer stem-like cells. The migrating cells represents non-cycling cells, thus some therapeutic approaches do not affect these subtypes. Additionally, autophagy is associated with CSCs and their therapy-resistance (Amaravadi et al., 2007; Lu et al., 2008; Nazio et al., 2019; Sosa et al., 2014). Altogether, disseminated tumour cells represent invasive and highly therapy-resistant subtype of tumour cells.

Additionally, disseminated tumour cells require a suitable niche for their growth. Multiple conditions, such as stemness, specific expression profiles, and senescent cell composition of the niche were suggested as important factors for efficient dissemination (Kaplan et al., 2005; del Pozo Martin et al., 2015). Additional factors are being evaluated.

This indicates that different conditions are needed for different tumour cells for efficient dissemination. Furthermore, both disseminated tumour cells and tumour cells at primary site provide stimuli which may regulate efficacy of the dissemination process as well (Hoshino et al., 2015).

2.8 Cancer recurrence

The therapy-surviving tumour cells or their subpopulations may promote tumour recurrence at the primary site. Furthermore, dissemination and metastatic diseases are also troublesome. Metastatic cells are highly invasive and therapy-resistant; they promote growth of micrometastases. However, the disseminated cells must undergo multiple complex, and thus hard to predict events, which may lead to overt metastases to occur years after initial diagnosis (Pan et al., 2017). Exposing mechanisms responsible for the switch of phenotype of metastatic cells would allow development of novel therapeutic strategies. Similarly, genes essential in these processes would enable better understanding of the events. Novel models of metastatic diseases are needed, since observations of the dissemination processes are highly stochastic *in vivo*. Additionally, therapy treatments potentiate the dissemination process (Anderson and Dische, 1981; von Essen, 1991; Martin et al., 2014; Moncharmont et al., 2014; Yuan et al., 2015); hence elucidating therapy-promoted changes in tumour cells and TME is of great importance.

2.9 Cancer dormancy

Metastatic disease may occur years after the diagnosis or treatment of the tumour. This is due to dormancy of tumour cells at secondary sites. Dormancy represents a highly resistant reversible non-cycling state of cells. The causes for cancer dormancy are still elusive.

In part, dormancy represents a feature of cancerous cells. However, dormant cancerous cells must overcome several issues associated with novel environment to promote the growth of micrometastases. Therefore, stimuli of pre-metastatic niche play crucial roles in exiting the state of dormancy (Bartkowiak et al., 2010; Hüsemann et al., 2008; Shiozawa et al., 2008; Sosa et al., 2014). The nutrients and oxygen supply must be provided to exit the dormant state of cancerous cells (Aguirre-Ghiso, 2007; Holmgren et al., 1995) Additionally, the dormant cells must overcome the immune editing (MacKie et al., 2003). The same is required for

micrometastases to grow into overt metastases. The immune editing is likely the main reason for late metastatic growth (MacKie et al., 2003), once the immune response is suppressed, the metastases may grow uncontrolled (Jimsheleishvili et al., 2014; Strauss and Thomas, 2010).

Three types of dormancy are defined: cellular, angiogenic and immune-induced dormancy (Aguirre-Ghiso, 2007). Cellular dormancy, an intrinsic program of the disseminated tumour cells, is sustained via multiple pathways. Additionally, this state is also regulated by outer stimuli provided by the environment. These include activation of p38 and Erk pathway in a ratio favourable for p38 pathway (Bartkowiak et al., 2010; Kobayashi et al., 2011; Masiero et al., 2011) (BMP7 mediated this effect via induction of p38 and via upregulation of p21 and p27 (Kobayashi et al., 2011)), suppression of Akt activity (Balz et al., 2012; Marshall et al., 2012), and activation of stress response in form of unfolded protein response (UPR) (Bartkowiak et al., 2010). Similarly to EMT, the dormant cancer cells are highly resistant to chemotherapy (Bartkowiak et al., 2009). This is likely mediated by stress response promoting autophagy, which is associated with therapy resistance (Lu et al., 2008; Sosa et al., 2014). CSCs also show elevated expression of ABC genes, responsible for drug efflux (Shervington and Lu, 2008). Additionally, the dormancy is also accompanied with the expression of stemness and quiescence factors (Adam et al., 2009). TGFβ2 signalling represents an additional stimulus for cancer dormancy (Bragado et al., 2013; Yumoto et al., 2016), but TGFβ3 and POSTN signalling were shown to awaken the dormant cells, thus promoting cell cycle entrance (Ghajar et al., 2013; Malanchi et al., 2012).

Causes for exit from cellular dormancy are not entirely clear. Once again, the environment certainly plays an important role, since TGFβ3 and POSTN signalling induced by endothelial cells of vascular vessels promoted proliferation in the dormant cells (Ghajar et al., 2013; Malanchi et al., 2012). The entrance of the dormant cells into cell cycle is also accompanied with increased expression of *VCAM1* (Lu et al., 2011) and activation of MYCK (Barkan et al., 2008). Why and how are these events induced is not elucidated, however, it was hypothesized that the dormant cells must overcome angiogenic dormancy prior to overcoming cellular dormancy (Sosa et al., 2014). Some speculate that therapy might promote genetic or epigenetic changes required for exit of cellular dormancy state (Goddard et al., 2018).

Theoretically, onset of immune system-mediated regulation of cancer dormancy follows angiogenic and cellular dormancy escape. After these events, proliferating disseminated cancer cells promote micrometastatic growth, whereas cytotoxic cells of the immune systems may eliminate disseminated cancerous cells, or equilibrium between cancer cell growth and cancer cell death is established. The cells of the immune system, predominantly CD8+ cytotoxic T lymphocytes and NK cells, are responsible for these events via IFNγ secretion and signalling. IFNγ induces G0 arrest in dormant cells via p27 (Farrar et al., 1999; Liu et al., 2017). Alternatively, CD4+ lymphocyte-mediated secretion of anti-angiogenic factors, CXCL9 and CXCL10, suppresses angiogenic events in the milieu (Müller-Hermelink et al., 2008).

The dormant cancer cells must overcome the immune surveillance to disrupt the equilibrium and establish an environment enabling their growth. Primary tumour mass, tumour cells, and associated non-cancerous cells of TME utilize multiple mechanisms responsible for evasion of the immune surveillance. However, mechanisms responsible for the immune evasion at secondary sites are described poorly. Cell lines derived from the dormant cells showed elevated expression of MHC I complexes (Mahnke et al., 2005), which allows evasion of NK cells-induced cell death, but not CD8+ lymphocytes induced-cell death. We can speculate that the disseminated tumour cells carry some of the genomic and epigenetic changes from primary tumour cells, which would allow CD8+ induced-cell death evasion or resistance, but these notions require further investigation.

Certainly, understanding the mechanisms of all types of dormancies is challenging, but necessary to eliminate the dormant cancer cells in patients. Several clinical trials have applied preventive treatment following primary tumour eradication, to eliminate or inhibit the growth of the dormant cancer cells (ClinicalTrials.gov identifiers: NCT03032406, NCT03400254, NCT01545648). Interestingly, one trial already showed markedly increased metastatic-free survival period, utilizing docetaxel following chemotherapy in breast cancer patients (Naume et al., 2014). These results indicate that dormancy must be counted with. A deeper understanding is crucial and will likely be beneficial.

2.10 Myeloid-derived suppressor cells

Among various immune suppressive cells of TME, MDSCs represent a very special compartment. MDSCs are relatively recently identified cells with a strong immune

suppressive capacity targeting predominantly T lymphocytes and NK cells. MDSCs promote tissue remodelling and neovascularisation, both processes associated with the tumour dissemination. Additionally, their abundance in patients' peripheral blood correlates with worsen prognosis and reduced therapy efficacy (Chen et al., 2014; Ostrand-Rosenberg and Fenselau, 2018).

Myelopoiesis-stimulating factors (e.g. GM-CSF, G-CSF, M-CSF) derived from the tumour mass stimulate expansion of myeloid precursors in the bone marrow (Bronte et al., 1999; Dolcetti et al., 2009). Subsequently, the myeloid precursors are targeted with pro-inflammatory stimuli derived from TME (Figure 4) (Bronte et al., 1999; Condamine et al., 2015; Dolcetti et al., 2009). This initiates immune-suppressive programs of expanded myeloid precursors, thus deriving MDSCs. MDSCs then migrate to the source of the pro-inflammatory stimuli, TME, to suppress these conditions. However, MDSCs suppress only the infiltrated lymphocytes and NK cells, but not the other sources of pro-inflammatory stimuli, thus the malignant milieu is reinforced and immune evasion is enabled (Gabrilovich et al., 2012).

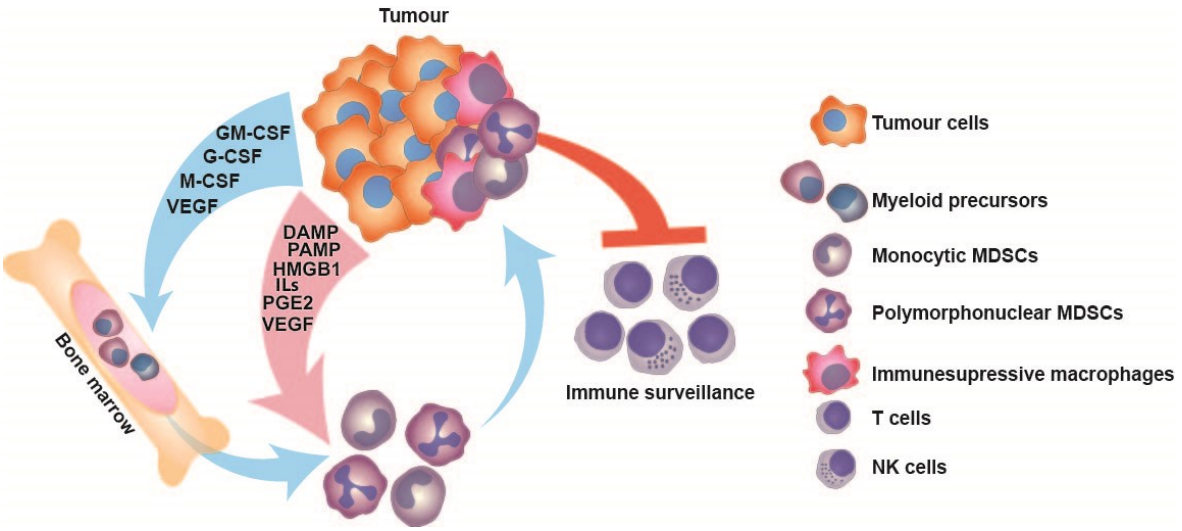


Figure 4. **A scheme of stimulation of myeloid-derived suppressor cells (MDSCs)**. Myelopoiesis-stimulating factors are released from the tumour mass (e.g. GM-CSF, G-CSF, M-CSF, and VEGFs) and travel into the bone marrow. Expanded myeloid precursors are released from the bone marrow and stimulated with pro-inflammatory stimuli (e.g. DAMPs, PAMPs, HMGB1, ILs, PGE2, VEGFs). Subsequently, their immune suppressive program is initiated, hence MDSCs are derived. MDSCs then migrate into the tumour mass and inhibit the immune surveillance. CSF – colony stimulating factor, GM – granulocytes/monocytes, G – granulocytes, M – macrophages, VEGF – vascular endothelial growth factor, DAMPs – damage-associated molecular patterns, PAMPs – pathogens-associated molecular patterns, HMGB1 - high mobility group box 1, ILs – interleukins, PGE2 – prostaglandin E_2. Adapted from (Pribyl, 2020).

In total, three subtypes of MDSCs are described. These are: polymorphonuclear MDSCs (PMN-MDSCs: morphologically resembling granulocytic myeloid cells, such as neutrophils), monocytic MDSCs (MN-MDSCs: resembling monocytes), and early-stage MDSCs (eMDSCs: resembling blast cells, monocytes, but also granulocytes). In human, MDSCs identification is relatively complicated. Usually, staining of surface markers is utilized (Bronte et al., 2016). In case of PMN-MDSCs, the staining of surface markers does not distinguish PMN-MDSCs from neutrophils. Therefore, ficoll gradient isolation is required. Following the procedure, the mononuclear fraction (the low-density fraction) contains PMN-MDSCs, whereas neutrophils are in the high-density fraction. However, this does not distinguish PMN-MDSCs from activated neutrophils. Such observations support a notion that PMN-MDSCs represent immune suppressive state of activated neutrophils (Pillay et al., 2013; Sippel et al., 2011), yet this needs further elucidation.

The immune suppressive capacity of MDSCs is mediated via multiple mechanisms from two main categories. These are: T cell receptor (TCR)-dependent and TCR-independent. This is based upon mechanisms of action. TCR-dependent mechanisms inhibit the function of TCR directly, thus reducing its antigen-recognizing potential. This is mediated via reaction of TCR with MDSCs-synthetized peroxynitrite ($ONOO^-$) (Nagaraj et al., 2007). Additionally, peroxynitrite efficiently nitrates CCL2 chemokine. Nitrilated CCL2 is not recognized by its original recipients, monocytes and T cells, but is an efficient chemokine of MDSCs (Molon et al., 2011).

TCR-independent mechanisms include secretion of cytokines (Gabrilovich et al., 2012; Sinha et al., 2007), such as IL-10 which polarizes macrophages into suppressive M2 phenotype. Reduction of cysteine and tryptophan availability in the milieu strongly affects the function of T cells. This is mediated via cytosine import and extracellular catabolism of tryptophan and arginine with IDO-1, or Arg-1 respectively (Rodriguez et al., 2004; Srivastava et al., 2010). Production of ROS is also utilized by MDSCs. Their actions promote reduction of TCR expression, hence T cell ability to recognize antigens is diminished (Corzo et al., 2009).

Additionally, MDSCs utilize cell-to-cell interaction to promote anergy in T cells. This is mediated mainly via PD-L1 induced actions (Iwata et al., 2016). MDSCs may also induce CD62L cleavage on the surface of T cells with ADAM17 protease, thus regulating ability of T cells to migrate into lymph nodes (Hanson et al., 2009).

Besides immune cells suppression, physiological function of MDSCs also lies in their ability to enhance the repair of damaged tissues. Extracellular matrix remodelling and induction of neovascularisations are two main features associated with tissue repair promoted by MDSCs. Secretion of matrix metalloproteinases and pro-angiogenic factors such as VEGFs promote these processes, but may be hijacked by tumour cells to promote their growth and spreading, which has been documented (Binsfeld et al., 2016; Johnson et al., 2018; Sinha et al., 2005; Yang et al., 2004).

Since MDSCs must pass from the peripheral blood into TME and their generation is directly regulated via tumour mass-secreted signals, their presence in the peripheral blood reflects the stage of progression. Indeed, breast carcinoma (Diaz-Montero et al., 2009), esophageal carcinoma (Chen et al., 2014), and thyroid cancer patients (Angell et al., 2016) showed elevated MDSCs level with the stage of progression. The overall survival of

melanoma patients was markedly reduced with elevated MDSCs peripheral blood levels (Jordan et al., 2013). MN-MDSCs specifically marked reduced survival rates in lymphomas (Marini et al., 2016; Wu et al., 2015) and multiple myeloma (Wang et al., 2015c). MN-MDSCs also markedly reduce chemotherapy efficacy in multiple myeloma (Lee et al., 2016) and Hodgkin's lymphoma (Romano et al., 2015). Additionally, elevated MDSCs levels predicted lower efficacy of ipilimumab in melanoma (Sade-Feldman et al., 2016) and MN-MDSCs marked the reduction of nivolumab efficacy also in melanoma (Weber et al., 2016).

Certainly, MDSCs represent detrimental components of TME responsible for its main features. In MDS, eMDSCs were shown to promote cell death of erythroid precursors, and thus promote pathological features of the disease (Chen et al., 2013). Therefore, eliminating MDSCs in MDS could alleviate from this symptom.

Altogether, MDSCs promote immune evasion, growth of tumour cells and facilitate their spreading. Targeting MDSCs with therapy has been proposed but results of several clinical trials attempting to eradicate MDSCs from TME are not satisfying (Winter et al., 2020). Furthermore, sole elimination of MDSCs does not prevent from their further accumulation, thus combination with elimination of other components of TME could provide more satisfying outcomes.

2.11 Myelodysplastic syndromes

A heterogeneous group of pre-leukemic diseases is known as myelodysplastic syndromes (MDS). Pro-inflammatory conditions in the bone marrow of MDS patients are established resulting in failure of the compartment (Sallman and List, 2019; Winter et al., 2020). Mutant clones of haematopoietic stem cells and progenitors (HSPCs) of myeloid lineage, so called blasts, thrive under inflammatory conditions (Muto et al., 2020). In general, blasts are recognized as non-differentiating cells of myeloid lineage, hence myeloid lineage of patients is strongly affected. Therefore, neutropenia, anaemia, and thrombocytopenia are common symptoms associated with the disease, and also main life-threatening aspects of the disease. Despite certain effort, blast mutations commonly associated with MDS struggle to make their way into clinics (Haferlach et al., 2014; Hasserjian, 2019). Blasts abundance in the bone marrow and cytogenetic profile of blasts are diagnostic criteria most

commonly utilized nowadays (Arber et al., 2016; Greenberg et al., 1997a; Winter et al., 2020).

Multiple criteria are used as diagnostic parameters. These include neutrophils count, haemoglobin concentration, and thrombocytes count, all measured in the peripheral blood of the patients. Next, the bone marrow biopsies reveal the blast count, which is a crucial determinant of patients' prognosis. And cytogenetic profile of the bone marrow cells is determined. Altogether, these numbers and the cytogenetic profile determine the prognostic score based upon which the patients are assigned into prognostic groups. With several updates, the most commonly used prognostic scoring systems are: Revised International Prognostic Scoring System (IPSS-R) (Greenberg et al., 2012) and World Health Organisation (WHO) classification (Arber et al., 2016). Nevertheless, which system is used is dependent upon clinicians; hence 1997 IPSS is sometimes also used (Greenberg et al., 1997b), despite its update in 2012. The patients corresponding to the low-risk groups are monitored or receive blood transfusions if necessary. The high-risk group patients receive chemotherapy, 5-azacytidine or 5-aza-2'-deoxycytidine. This has not changed for last 20 years, since no better treatment has passed clinical trials. The only exception is lenalidomide, which is utilized for MDS 5q- syndrome patients (Krönke et al., 2015). Usually, this group of patients has better prognosis.

Together with blasts' accumulation, the bone marrow milieu changes (Barreyro et al., 2018; Sallman and List, 2019; Winter et al., 2020). Repercussions of these changes may eventually lead to leukemic transformation, most commonly into acute myeloid leukaemia (AML) or chronic myelomonocytic leukaemia (CMML). In clinics, AML is considered when patients' bone marrow blasts reaches >20% or >25%, hence definition of AML differs according to the prognostic score applied. MDS median survival varies according to patients corresponding prognostic group. The low-risk group has 5.7 years median survival, the high-risk group have 5 months median survival according to IPSS (Greenberg et al., 1997a). Additionally, 25% of patients transform into AML. In elderly AML patients, median survival is 6 months when chemotherapy is utilized. Without chemotherapy, AML median survival is 2 months (Oran and Weisdorf, 2012). If haematopoietic stem cells transplantation is an option, the survival is usually extended by several months, in the high-risk MDS (Beran, 2000) and AML (Oran and Weisdorf, 2012).

2.11.1 5-azacytidine and 5-aza-2'-deoxycytidine in MDS and AML

5-AC and 5-dAC were used in various clinical trials. However, for the purpose of this introduction; we refer to studies which we consider to be related to the topic of this thesis.

Several clinical trials applied 5-AC as a monotherapy in MDS. These trials applied varying dosing of 5-AC: 75 mg/m^2 daily for 7 days (Silverman et al., 1993) with 12% complete remission, 25% partial remission. In a subsequent study the high-risk MDS patients received 5-AC, 75 mg/m^2 seven times daily every 4 weeks (99 patients), versus non-treated patients (92 patients) in 191 patients with the high-risk MDS. In this trial, 6% complete remission was achieved, 10% partial remission, 47% haematological improvement (Silverman et al., 2002). Median for leukemic transformation was 22 months *vs*. 12 months, and median survival 18 months *vs*. 14 months. These studies contributed most significantly in application of 5-AC as a first-line treatment for MDS (Kaminskas, 2005).

Alterations in 5-AC dosing applying 40 to 50 mg/m^2 given over 24 hours for three days every 6 weeks (Wijermans et al., 1997) and later with 15 mg/m^2 given over 4 hours every 8 hours for 3 days every 6 weeks (Wijermans et al., 2000) (in both cases, the total dose was 120 to 150 mg/m^2 per course). The response rates were similar for the trials reaching 50% response rate. Newer studies correspond to these pioneering results (Santini, 2019). In the next trial, 20% of patients showed complete remission and a median survival reached 19 months in Int-1 group *vs.* 15 months control group. This was not achieved in the high-risk groups (Wijermans et al., 2000).

Haematopoietic stem cells transplantation is the only curative option for eligible MDS patients, with three-years relapse free survival in 24% of MDS patients (Festuccia et al., 2017). This is reduced in patients with *TP53, ASXL1,* or *RUNX1* mutation (Della Porta et al., 2016). Patients with *TP53* mutation showed 30% relapse-free survival in one-year period and only 10% in 3-year period (Della Porta et al., 2016).

5-dAC induced expression of immune checkpoint ligands by CD34+ blasts from MDS and AML patients (Øyan et al., 2005) and the high-risk MDS possessed higher levels of CTLA-4, PD-L1, and PD-L2. The expressions of these genes were elevated with 5-dAC treatment (Yang et al., 2014). Relatively high immune checkpoint expression is likely responsible for CD8+ T cells exhaustion in high-risk MDS patients. Hence, T cells of these patients could be more prone to ICI therapy. However, single therapy ICI showed low

efficacy in AML and MDS patients (Garcia-Manero et al., 2016), but anti-CTLA-4 showed effect in 5-dAC treated refractory MDS patients (Garcia-Manero et al., 2016). Therefore, combination of ICI with 5-dAC is of interest (Daver et al., 2018). Yet deeper understanding of the bone marrow milieu of MDS is essential.

2.11.2 The bone marrow of MDS

Haematopoiesis takes place in the bone marrow, where HSCPs reside in stem niches. Vascular, reticular, and endosteal niches are responsible for regulation of HSCPs expansion, differentiation, and renewal, but also for retaining their quiescence. These effects are mediated via complex milieu of cytokines and other signalling molecules creating a strictly regulated yet responsive environment of the bone marrow (Ghobrial et al., 2018).

Inflammation is a detrimental feature of ongoing MDS (Figure 5). However, the causes for aberrant milieu are not clearly elucidated. Similarly to the inflammatory response in solid tumours described in previous chapters, the inflammation could be initiated via autoimmune response, inflammaging, or stimulation of innate immune response induced with bacterial components (Braun and Fenaux, 2013; Sallman and List, 2019), yet a clear evidence is missing. Despite being documented as important mediators of the bone marrow changes in MDS, mutations commonly associated with MDS play only partial role in these alterations. Mutations of splicing factors machinery may promote changes in blast gene expression enhancing the inflammatory response of malignant clones (Liang et al., 2018); however, mutations themselves might not be detrimental during the onset of the disease (Jaiswal and Libby, 2020; Muto et al., 2020).

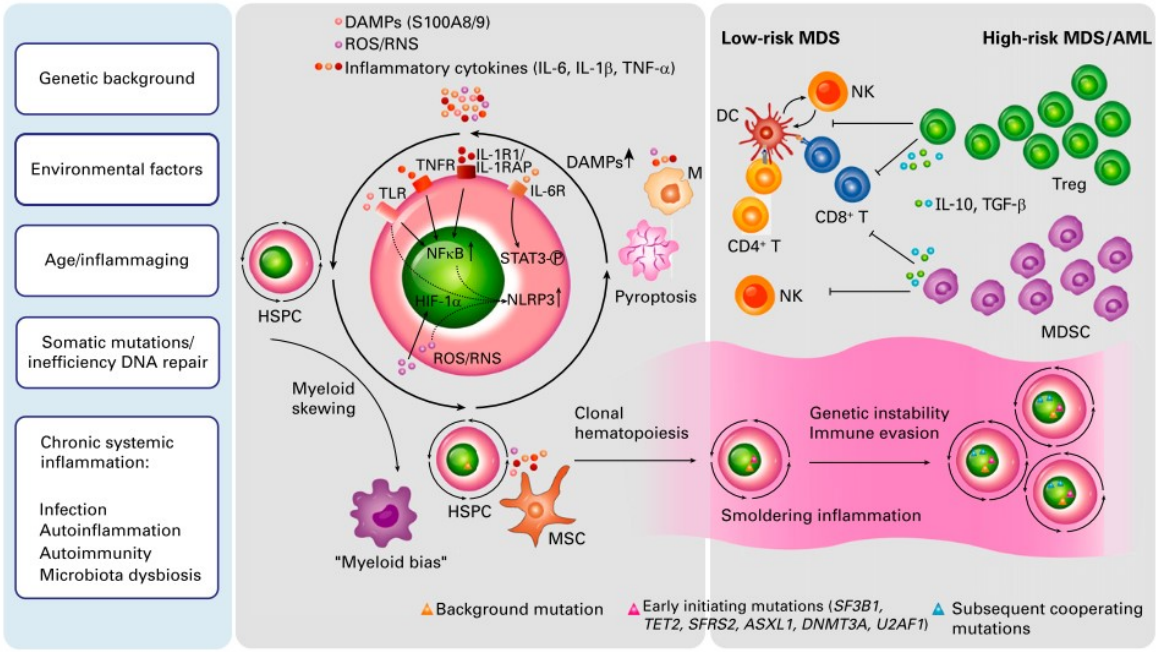


Figure 5. **Progression of changes in the bone marrow of MDS.** Chronic inflammation in the bone marrow is promoted by different sources. Mediators of inflammation are inflammatory molecules (DAMPs, ROS/RNS) and inflammatory cytokines (IL-6, IL-1β, TNF-α, etc.). Chronic exposure to these molecules promotes aberrant signalling in HSPCs induced via TLR, TNFR, IL-1R1/ IL1RAP receptors. This may lead to pyroptosis (inflammatory cell death), myeloid skewing, induction and accumulation of mutations in various genes, resulting in clonal haematopoiesis. The inflammatory conditions are enhanced with pyroptosis and the inflammatory molecules generated by mutant HSPCs and immune response in the bone marrow. Eventually, anti-inflammatory milieu is established with strong immune suppressive actions of MDSCs and Tregs, altogether enabling immune evasion and accumulation of the malignant clones, thus resulting in the leukemic transformation. HSPC – Haematopoietic stem cells and progenitors, MSC – mesenchymal stem cells, DAMPs – damage-associated molecular patterns, ROS – reactive oxygen species, RNS – reactive nitrogen species, IL – interleukins, TNF-α – tumour necrosis factor α, TGF-β – tumour growth factor β, M – macrophages, TLR – Toll-like receptors, TNFR – TNF receptor, IL-1R1 – Interleukin-1 receptor 1, IL-1RAP – Interleukin-1 receptor associated protein, IL-6R – interleukin 6 receptor, DC – dendritic cells, NK – natural killer cells, T – T cells, Treg – regulatory T cells, MDSCs – myeloid-derived suppressor cells, MDS – myelodysplastic syndromes, AML – acute myeloid leukaemia. SF3B1 – splicing factor 3B subunit 1, TET2 – tet methylcytosine dioxygenase 2, SFRS2 – splicing factor, arginine/serine-rich 2, ASXL1 – additional Sex Combs Like 1, DNMT3A – DNA methyltransferase 3 alpha, U2AF1 – U2 small nuclear RNA auxiliary factor 1. Adapted from (Winter et al., 2020).

Indeed, clonal haematopoiesis of indeterminate potential (CHIP) is an observation with 10% prevalence in >70 years old individuals. The bone marrow of individuals with CHIP contains HPSCs with mutation or mutations commonly associated with MDS,

however, these individuals do not manifest the symptoms of MDS (Bewersdorf et al., 2019). Therefore, the blast mutations are the feature of MDS, but the onset of the disease is stimulated via different stimuli. In recent work, the authors showed that HPSCs clones overexpressing *TRAF6*, which is often overexpressed as a result of mutations in HSPCs in MDS, show no growth advantage in normal bone marrow. However, once inflammatory stimuli are introduced into the bone marrow then *TRAF6*-overexpressing clones thrive (Muto et al., 2020). These results indicate that chronic inflammation is likely an essential factor during the onset of the disease. Once established, inflammatory signalling promotes changes in the bone marrow, including accumulation of MDSCs and other cell types (Winter et al., 2020), which altogether initiate stress response resulting in pro-inflammatory cell death and oxidative stress, both possibly causing DNA damage and the increase in mutational burden of blasts associated with MDS progression (Chen et al., 2013; Guida et al., 2014; Yang et al., 2015).

The role of MDSCs in pathogenesis of MDS is crucial. MDSCs promote pyroptosis in HSCPs (Chen et al., 2013), which maintains pro-inflammatory milieu in the bone marrow and enhances their accumulation. However, the function of MDSCs is to inhibit the immune response. In doing so, accumulated MDSCs promote anti-inflammatory conditions; hence the bone marrow of the high-risk MDS is highly immunosuppressive. This enables the accumulation of malignant blasts and subsequent leukemic transformation (Winter et al., 2020; Yang et al., 2015). MDSCs promote their function via secretion of small molecules, intercellular contacts, and cytokine signalling. Hence to understand the pathological changes of the bone marrow in MDS, the role of MDSCs and their secreted components might provide important answers.

2.12 Suprabasin

Suprabasin (*SBSN*) represents a recently identified oncogene of unknown function. Since *SBSN* is not a well-studied gene, its role in physiology and pathology is largely undiscovered. It is proposed that *SBSN* encoded proteins are secreted signalling molecules, yet the receptor(s) are unknown. *SBSN* is associated with cancer, but causes of its aberrant expression are yet to be uncovered.

SBSN is physiologically expressed in stratified epithelia in both mice and human. Skin is the main expression site of *SBSN* (Matsui et al., 2004; Park et al., 2002), but other

organs with SBSN expression are also described. These include thymus, tonsils, esophagus, uterus, and vagina. Additionally, *SBSN* was shown to be expressed or elevated in various tissues following stress events, these include, for instance, astrocytes of human brain following lipopolysaccharide (LPS) treatment (Ichinose et al., 2018) or murine urothelial cells in close proximity of intracellular uropathogenic *Escherichia coli* colonies (Reigstad et al., 2007). Both *SBSN*-inducing stimuli suggest that innate immune response could be at least partially responsible for elevated *SBSN* expression. Interestingly, muscle biopsies of exercising alaskan dogs showed elevated *SBSN* mRNA levels, as well as mRNAs of genes corresponding to inflammatory response (Brass et al., 2009). These examples seem rather obscure, yet another study showed elevated *SBSN* mRNA levels in carotid arteries of sheep exposed to high altitudes, hence long-term hypoxia resulting in acclimatization (Goyal and Longo, 2014). Authors noted that among other 57 elevated mRNA genes, some of them are targets of LPS-induced signalling (Goyal and Longo, 2014). Altogether, inflammatory response might play a role in *SBSN* expression under pathological conditions.

Inflammatory signalling, as already described, is an essential factor in tumour progression and many aspects associated with tumour diseases. Hence it is no surprise that *SBSN* is upregulated also in cancer. Increased *SBSN* expression was shown in esophageal squamous cell carcinoma (ESCC) (Zhu et al., 2016) and tumour endothelial cells of colon cancer (Alam et al., 2014). *SBSN* transcript levels were elevated in NSCLC (Glazer et al., 2009), ESCC (Zhu et al., 2016), and metastatic head and neck cancer cells (Liu et al., 2019). Next, *SBSN* expression was also observed in *in vitro* cancer models, such as glioblastoma (Formolo et al., 2011) and salivary gland cancer cell lines (Shao et al., 2012), where its expression was associated with elevated invasiveness, and anchorage-independent growth, respectively. Last but not least, oral squamous cancer cells elevated *SBSN* mRNA following treatment with *Porphyromonas gingivalis* lysate (Liu et al., 2020), once again supporting the role of innate immune response and inflammation in regulation of *SBSN* expression. Note, in the later study, *P.gingivalis* infection was shown to be responsible for immune evasion of the cancerous cells.

Human *SBSN* encodes three protein isoforms (Figure 6). All represent secreted molecules. Human isoform-2 is a fully spliced version, isoform-1 contains intron-1 retention, and isoform 3 is missing exon-2. Intron-1 retention in SBSN-1 encodes a highly conserved domain containing Ala-/Gly-/His-rich repeats. In general, repetitive regions are

associated with mechanically resistant proteins, hence corneocyte-expressed *SBSN* proteins were proposed as important components of cornified envelope, the outer structure of corneocytes essential for the function of skin (Park et al., 2002). Yet, this role was not clearly supported by other studies.

Human SBSN isoform-2 is the isoform associated with oncogenic role of *SBSN* (Takahashi et al., 2020; Zhu et al., 2016). Importantly, mouse isoform corresponding to human SBSN isoform-2 has not been identified (*Mus musculus*), thus mouse probably lacks this isoform (Hunt et al., 2018; Park et al., 2002). This is an important observation when comparing human and mouse *SBSN* functions. Both human and mouse possess isoform-3, but this isoform is the least studied one.

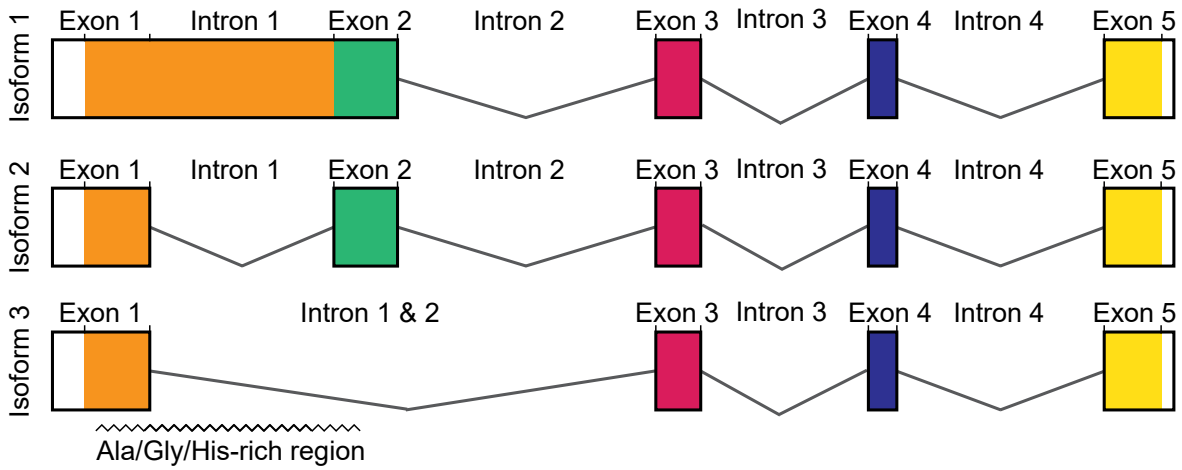


Figure 6. **Transcripts of human *SBSN* isoforms**. Human *SBSN* gene encodes three isoforms. The longest isoform-1 retains intron-1, this results in retention of a long repeat containing Ala/Gly/His-region. Isoform-2 is associated with the oncogenic potential of *SBSN*. This isoform is missing in *Mus musculus*. Isoform-3 lacks exon-2 and is the least studied isoform of *SBSN*. White boxes mark untranslated regions. Ala – alanine, Gly – glycine, His – histidine.

SBSN was shown to stimulate several signalling cascades. SBSN-2 induced Wnt pathway via phosphorylation of GSK3β and subsequent β-catenin stabilization in ESCC (Zhu et al., 2016). This study originally proposed its role as an oncogene, since SBSN-2 expressing tumours were larger and markedly more vascular. SBSN-2 treatment induced HUVEC sprouting and migration via activation of p38 pathway, but Akt also showed elevated phosphorylation, yet it was not essential for the observed phenotype (Takahashi et al., 2020). *SBSN* was also expressed in tumour endothelial cells (TEC) of

colon cancer, which further supports its association with vascular changes in cancer (Alam et al., 2014). However, in TEC, this effect was mediated via Akt phosphorylation.

Description of *SBSN* in this chapter provides information necessary to understand the results of two research articles which are parts of this thesis. We complied all current knowledge of *SBSN* in a yet to be published review. For the interested reader, we provide this review at the end of this work.

Certainly, *SBSN* holds a great undiscovered potential. It is associated with cancer and inflammation and promotes activation of multiple signalling pathways responsible for survival, differentiation, and importantly, therapy resistance. A lot remains to be uncovered in search for the role of *SBSN* in cancer progression. We hope our studies presented in this thesis will help in these attempts.

3. Aims of the study

The thesis contains two research articles. In these we attempted to elucidate several topics.

In the first research article we aimed to:

- Describe the features of therapy-surviving cancer cells with low-adherent phenotype induced with clinically relevant radiotherapy and chemotherapy regimes in *vitro*.
- Elucidate the role of *SBSN* in low-adherent therapy-resistant cancer cells.

In the second article we aimed to:

- Identify the prognostic potential of *SBSN* expression in the bone marrow of myelodysplastic syndromes patients.
- Identify the source of *SBSN* expression in the bone marrow of myelodysplastic syndromes patients.

The connection of both topics depicted in the research articles is explained in a chapter separating the articles.

4. List of utilized methods

Here is a list of individual methods utilized in both articles presented below. The descriptions of the methods are within method sections of particular articles.

- Cell cultures
- Colony-forming assay
- Detection of apoptosis using fluorescence activated cell sorting (FACS)
- Dual-luciferase reporter assay
- Enzyme-linked immunosorbent assay
- Fluorescence-activated cell sorting
- Generation of the stable SBSN-expressing cell line via a lentiviral system
- Human bone marrow and peripheral blood plasma samples preparation
- Immunohistochemistry
- Indirect immunofluorescence
- Isolation of bone marrow mononuclear cells
- Magnetic-activated cell sorting (MACS)
- Molecular cloning
- Mouse spontaneously metastasizing 4T1 mammary gland cancer cell line
- Proteomic analysis
- Quantitative real time PCR (RT-qPCR)
- Gene knockdown with RNA interference
- SDS/PAGE and immunoblotting
- Transient transfection
- Whole-genome expression array

5. The first article

The first article is entitled: “Interferon-regulated suprabasin is essential for stress-induced stem-like cell conversion and therapy resistance of human malignancies” (Hubackova et al., 2019).

The article has been published in 2019 in *Molecular Oncology* (2019 2-Year Impact Factor: 6.574).

Authors: This article has shared co-first authorship. The first authors are Soňa Hubáčková (SH), Miroslav Přibyl (MP), and Lenka Kyjacová (LK). Both SH and LK started the work and contributed in first 2-3 years (approximately 2013-2016). Subsequently, MP contributed in following 3 years (2016 – 2019).

Summary: Work presented in the article follows up a previous article by Kyjacova *et al*. In the previous work, authors utilized prostate cancer models irradiated with clinically relevant radiation dosage to induce therapy-resistant fraction of cancer cells with low-adherent phenotype. These anoikis-resistant non-cycling cells expressed markers of stemness and required active Erk and Akt signalling for their survival (Kyjacova et al., 2014).

In the following study presented here (Hubackova et al., 2019), we expanded the knowledge of therapy-induced low-adherent cells. We used additional model cell lines and showed that besides radiotherapy, 5-AC also induced the similar phenotype. The expression profiles of low-adherent cells showed hundreds of dysregulated genes, including upregulation of genes responsive to IFN treatment. Indeed, IFNγ treatment induced low-adherent phenotype as well. Importantly, *SBSN* was among the upregulated genes, and we showed, that its expression is essential for low-adherent cells, since downregulation of *SBSN* expression resulted in decrease of low-adherent cells abundance following the therapy treatments.

MP contribution: MP performed experiments to obtain data presented in these figures: Figure 3I, J; Figure 4G, H, I; Figure 5E, F, G; Figure 6A (electrophoresis was performed by SH), B, C, D; Supplementary Figure 1B; Supplementary Figure 3A, B; Supplementary Figure 4E, F, G, H, I; Supplementary Figure 5E. MP also assisted with writing the article.

6. Linking the publications

In the first publication (Hubackova et al., 2019) we showed, that 5-AC treatment promotes therapy-resistant phenotype of cancer cells. 5-AC-induced *SBSN* and downregulation of *SBSN* expression reduced low-adherent cells abundance, suggesting its role in promoting the phenotype or survival of low-adherent cells.

Since 5-AC is the first-line treatment in the high-risk MDS, we asked whether 5-AC induces *SBSN* in the bone marrow of MDS patients and what would be the effect of this change.

Indeed, we showed that *SBSN* is markedly elevated in the bone marrow of a subgroup of high-risk MDS patients. Importantly, *SBSN* expression was mediated by MDSCs and not by 5-AC-therapy (Pribyl et al., 2020). Results and discussions are presented in following chapters.

7. The second article

The second article is entitled: “Aberrantly elevated suprabasin in the bone marrow as a candidate biomarker of advanced disease state in myelodysplastic syndromes” (Pribyl et al., 2020).

The article has been published in 2020 in Molecular Oncology (2019 2-Year Impact Factor: 6.574).

Authors: MP is the first author of this article.

Summary: Following the previous study (Hubackova et al., 2019), we asked whether MDS patients show elevated SBSN in the bone marrow. Importantly, 5-AC is used as the first-line treatment for the high-risk MDS and 5-AC induced the expression of *SBSN in vitro* (Glazer et al., 2009; Hubackova et al., 2019). Indeed, *SBSN* was elevated in the bone marrow of MDS, but this was not caused by 5-AC therapy. Hence, a cellular compartment is responsible for the expression. Indeed, *SBSN* aberrant expression was mediated by MDSCs in the bone marrow of a subgroup of the high-risk MDS patients. Additionally, elevated SBSN levels negatively correlated with T cells abundance and CCL2.

MP contribution: The experiments in this publication were predominantly performed by MP. Except for SBSN, other cytokines and cell counts in patient cohort #3 were measured by Alena Moudra (AM) in her previous study (Moudra et al., 2016). SH performed experiments for Figure 1A and 1B. MP and Zdeněk Hodný wrote the article.

8. Discussion

8.1 Therapy promotes therapy-resistant phenotype

Metastatic diseases are responsible for most of the cancer-caused deaths. Upon overcoming the state of dormancy, disseminated tumour cells promote growth of micrometastases which may turn into overt metastases. Adaptive non-mutational mechanisms are also induced with therapy. These may be responsible for cancer evolution (Russo et al., 2019), resistance (Pisco et al., 2013), spreading (Aldonza et al., 2020), and subsequently enabling cancer recurrence. Our results showed that the commonly used cancer therapy treatments, such as fractionized irradiation and 5-AC chemotherapy promoted therapy-resistant non-cycling anoikis-resistant phenotype of cancer models *in vitro*. We refer the cells with this phenotype as 'low-adherent cells'. Importantly, we observed that low-adherent cells are able to reattach and re-enter the cell cycle which resulted in growth of colonies. Thus partially resembling the events of tumour dissemination proposed *in vivo*.

Dormancy is a non-proliferative state; this means that the dormant tumour cells do not promote the growth of metastases unless the dormancy state is overcome. Three types of dormancy states are described (angiogenic, immune, and cellular dormancy). Novel blood vessels provide nutrients and oxygen supply for tumour cells. Subsequently, the requirements for angiogenic dormancy exit are met. Upon immune suppression, immune dormancy is passed (Jimsheleishvili et al., 2014; Strauss and Thomas, 2010). Immune suppression is mediated via immune suppressive cells activity (e.g. MDSCs), but possibly by tumour cells as well. Cellular dormancy remains the least understood type of described dormancies. Certainly, surpassing angiogenic dormancy was proposed to enable surpassing cellular dormancy as well (Sosa et al., 2014). Since cellular dormancy is likely an intrinsic program of dormant tumour cells, models to study this phenomenon would be useful.

Altogether, we showed that both irradiation and 5-AC therapy, both commonly utilized in cancer treatment, promote therapy-resistant dormant-like state of cancer cells. This model could provide useful knowledge of cellular dormancy, the least understood type of dormancy.

8.2 IFN pathway is essential for therapy-resistant phenotype

Besides previously observed features of dormant tumour cells, such as therapy resistance, inhibited proliferation, and stemness, low-adherent cells showed increased expression of MHC I components (namely HLA-C, E, G), which has been supported previously (Mahnke et al., 2005). Additional IFN-responsive genes' transcripts were elevated, suggesting IFN pathway is induced in low-adherent cells. Indeed, IFNγ treatment, but not IFNβ, promoted low-adherent phenotype, similar to irradiation or 5-AC described above. This suggests that IFN pathway is essential effector pathway for all three treatments inducing low-adherent cells. This supports the notion presented here that irradiation, 5-AC, or IFNγ treatments induce the same phenotype. Additionally, Erk inhibition resulted in reduction of low-adherent cells abundancy as well, which indicates that IFN pathway induces Erk signalling, suggesting that Erk pathway is also essential in the development of low-adherent phenotype.

IFNγ signalling induces MHC I components expression, enforcing cells to present antigens (Basham and Merigan, 1983; Kalbasi and Ribas, 2020). This could potentiate clearance via immune cells. Conversely, mechanisms enabling immune evasion are also promoted via IFNγ signalling, such as PD-L1 upregulation (Bellucci et al., 2015; Garcia-Diaz et al., 2017).

IFNγ is essential in anti-tumour activity; hence entering the state of dormancy could represent a mechanism of resistance of cancer cells against immune surveillance-promoted clearance. The response of tumour cells to IFNγ resulting in immune suppression and evasion has been documented. IFNγ promoted cell cycle arrest, quiescence, and dormancy, or dormant-like states (Bosserhoff et al., 2004; Liu et al., 2017; Müller-Hermelink et al., 2008). Besides, chronic stimulation with IFNγ resulted in ICI therapy resistance as well as radiotherapy resistance (Benci et al., 2016).

5-AC inhibited proliferation and promoted a dormant-like state in cancerous cells, including CSCs (Tsai et al., 2012). Additionally, 5-dAC induced IFN response in colon cancer models (Karpf et al., 1999), as well as MHC I glycoproteins expression (Rhee et al., 2002). Subsequent studies confirmed IFN pathway stimulation in NSCLC models following the treatment with 5-AC (Wrangle et al., 2013). Therefore, 5-AC likely promotes dormancy via IFN pathway.

These results indicate that irradiation, 5-AC, or IFNγ induce Erk and IFNγ signalling. Both pathways are essential for the phenotype, since their inhibition disrupts it.

8.3 *SBSN* expression is essential for low-adherent cells

Among hundreds of differentially expressed genes we selected *SBSN* to investigate more deeply. *SBSN* is a proposed oncogene, which is suggested by several reports (Takahashi et al., 2020; Zhu et al., 2016). Aberrant expression of *SBSN* is associated with cancer, specifically with invasiveness of glioblastoma cell line (Formolo et al., 2011), anchorage-independent growth *in vitro* (Shao et al., 2012), or enhanced proliferation *in vitro* and *in vivo* (Zhu et al., 2016). Besides that the knowledge of *SBSN* is lacking. Importantly, the oncogenic role of *SBSN* was attributed to its isoform-2 (Zhu et al., 2016).

Induction of *SBSN* transcript with 5-AC was documented (Gaykalova et al., 2012). We showed that *SBSN* expression is induced with irradiation, 5-AC, or IFNγ treatments. This has not been documented before. Erk pathway promoted the expression of *SBSN* and Erk inhibition suppressed the expression. BORIS was shown to promote *SBSN* transcripts elevation (Gaykalova et al., 2012) and 5-AC induced BORIS levels (Karpf, 2006), hence 5-AC induced *SBSN* expression via BORIS in our model is also likely.

In low-adherent subpopulation of therapy-surviving cells, we observed SBSN isoforms with ~70kDa. This suggests SBSN-1 is induced in low-adherent cells. One report indicates, that SBSN proteins may undergo posttranslational modifications including cross-linking mediated by transglutaminases 2 and 3 (Park et al., 2002). Hence, we cannot exclude other possibilities, such as SBSN-2 crosslinking and other cross-reactions. Nevertheless, we also cannot exclude a possibility of functional difference of individual isoforms. SBSN-2 promotes proliferation; however SBSN-1 might promote therapy or anoikis resistance, both the features of our model. Indeed siRNA-mediated knockdown of *SBSN* resulted in markedly reduced numbers of low-adherent cells following their induction. This suggests that *SBSN* is essential to manifest the therapy-induced low-adherent phenotype of cancer cells.

Indeed, *SBSN* expression induced Akt, Wnt, or p38 pathways in various models. Wnt signalling is associated with therapy-resistance and cancer stemness (Malanchi et al., 2012; Pisco et al., 2013; Vermeulen et al., 2010; Wang et al., 2015b) and p38 is crucial for

maintaining cellular dormancy (Balz et al., 2012; Bartkowiak et al., 2010; Gao et al., 2017; Kobayashi et al., 2011; Marshall et al., 2012; Masiero et al., 2011). Hence activation of Wnt and p38 pathway via SBSN could explain these features observed in low-adherent cells. These require further investigation.

8.4 *SBSN* is elevated in tumour samples and cancer tumour cells

Aberrant expression of *SBSN* was documented in several tumour types, such as NSCLC (Glazer et al., 2009), salivary gland adenoid cystic carcinoma (Shao et al., 2012), and ESCC (Zhu et al., 2016). *SBSN* was also aberrantly elevated in TEC of renal cancer and colon cancer where it induced Akt activity (Alam et al., 2014).

We supported previously documented increase of *SBSN* mRNA levels in colon cancer (Alam et al., 2014). Importantly, we identified increased *SBSN* mRNA levels in large number of ovarian carcinoma samples. Additionally, SK-OV-3 ovarian carcinoma cell lines showed *SBSN* expression under standard conditions.

As we proposed, *SBSN*-expressing cells with low-adherent phenotype resemble cellular dormancy. Interestingly, spontaneously metastasizing murine mammary carcinoma 4T1 model elevated *SBSN* mRNA levels in circulating tumour cells. Circulating tumour cells travel through bloodstream prior settling in a metastatic niche, hence these results further support the role of *SBSN* in dormancy and tumour dissemination.

8.5 *SBSN* is elevated in MDS patients

5-AC therapy has been a first-line treatment in MDS for multiple years. Unfortunately, only 50% of patients respond to the therapy (Santini, 2019). In our previous study, we showed that 5-AC induced therapy-resistant phenotype. Since these therapy-resistant cancer cells required *SBSN* expression for their survival, we asked whether *SBSN* is expressed in the bone marrow of MDS patients and whether the therapy affects these changes.

We observed elevated *SBSN* mRNA levels in MDS patients as well as elevated *SBSN* protein levels in the bone marrow plasma samples of MDS patients. We compared *SBSN* mRNA or protein levels between two groups of patients, the patients undergoing

5-AC therapy and patients without the therapy. Interestingly, no significant difference in *SBSN* expression between these groups was observed. This indicates that 5-AC therapy does not markedly affect the *SBSN* expression in the bone marrow of MDS patients. Therefore, *SBSN* expression is inherent with pathophysiology of MDS.

8.6 SBSN in MDS patients' stratification

In a recent review publication (Winter et al., 2020), authors stressed that failures of clinical trials in MDS are likely caused by insufficient stratification of patients. This means that the syndromes themselves are more diverse than the current stratification of patients allow. Indeed, every patient is different, but based upon current stratification systems we can assume that within the prognostic groups are subgroups with varying predispositions towards treatments. A group of patients with the same prognosis might be recruited into a trial, but this group is consisted of patients with varying bone marrow environments and varying mutations, both affecting the disease progression and response to the treatment. So far, only two mutated genes made it into IPSS-R clinical stratification, these are *SF3B1* and *TP53* (Haferlach et al., 2014; Hasserjian, 2019). Despite the identification of various mutations, no other showed prognostic potential. Additionally, biomarkers in MDS have not been identified. Thus identification of biomarkers would allow better stratification. Subsequently, this would provide more accurate recruitments into clinical trials and identification of efficient therapies for specific subgroups.

Upon assigning our cohort of patients into their prognostic groups we noticed that patients with the highest SBSN levels in the bone marrow were predominantly poor-prognosis patients (Int-2 according to IPSS, and EB-2 according to WHO classification), but not all poor-prognosis patients showed elevated SBSN levels. This indicates that a subgroup of poor-prognosis MDS patients has elevated *SBSN* protein levels. Interestingly, AML patients showed SBSN levels comparable to healthy donors or the low-risk MDS patients, indicating that *SBSN* expression is lost following the leukemic transformation. Indeed, in a group of three poor-prognosis MDS patients, we showed that following the transformation into AML, SBSN levels were markedly reduced. Hence, *SBSN* upregulation is a feature of a specific poor-prognosis subgroup prior the leukemic transformation.

Additionally, *SBSN* protein levels in the peripheral blood corresponded to *SBSN* protein levels in the bone marrow. Since the bone marrow biopsies are currently the only option in determining the patients' progression, SBSN levels in the peripheral blood represent an accessible biomarker of the disease. Whether SBSN levels in the peripheral blood could be utilized as markers of the disease progression require further investigation.

8.7 *SBSN* is expressed by MDSCs in the bone marrow of MDS

To identify the cellular source of *SBSN* expression in the bone marrow of MDS, we performed immunohistochemistry and immunofluorescence staining of SBSN in the bone marrow sections and smears. We also performed fluorescence-activated cells sorting to isolate specific subpopulations from the bone marrow of MDS and control group patients. Utilizing these techniques we identified a specific myeloid compartment, MDSCs, as a cellular source of *SBSN* expression in the bone marrow of MDS.

Interestingly, eMDSCs and PMN-/MN-MDSCs showed the highest levels of *SBSN* mRNA in MDS. This indicates that MDSCs are the source of aberrant expression of *SBSN* in the bone marrow of MDS. Monocytes and neutrophils-containing ficoll-isolated high-density fraction showed lower fold change levels of *SBSN* mRNA. These cells are likely responsible for basal SBSN levels observed in control groups and low-risk MDS. Importantly, abundance of eMDSCs, PMN-/MN-MDSCs, or monocytes was not significantly different between MDS and control groups. Hence, elevated *SBSN* expression is not mediated by increased MDSCs numbers, but rather by elevated stimuli inducing the expression in MDSCs. Identifying the stimuli responsible for these observations require further effort.

8.8 SBSN and immune system

Our results indicate that *SBSN* is expressed by MDSCs in the bone marrow of MDS. These cells represent an immune suppressive compartment. MDSCs are associated with cancer; however their role in pathobiology and physiology is also crucial. For example they play an important role in pregnancy; where MDSCs prevent T cells reaction towards foetus, thus establishing the tolerance (Bartmann et al., 2016; Kang et al., 2016; Nair et al., 2015). Importantly, MDSCs are detrimental in MDS pathogenesis. MDSCs-secreted S100A8/9

alarmins induced pro-inflammatory cell death of erythroid precursors (Chen et al., 2013). This results in reduced development of erythrocytes, thus causing anaemia and increase inflammation in the bone marrow, which significantly contributes to the disease pathogenesis and progression.

S100A8/9 represents innate immune response proteins commonly elevated following bacterial infection (Wang et al., 2018). *SBSN,* as we suggest, is likely inflammation and/or innate immune responsive gene, since bacterial components induced *SBSN* mRNA levels. We cannot state what stimuli are promoting *SBSN* expression in MDSCs yet, but these stimuli are certainly changing among various patients, thus altering the level of *SBSN* expression.

We can only speculate what signals promote *SBSN* expression in MDSCs. The bone marrow milieu of MDS undergoes dramatic changes during the progression of the disease. Levels of various cytokines shift; cellular composition affects and promotes these changes as well as therapy (Moudra et al., 2016; Winter et al., 2020; Yang et al., 2015).

The changes in cytokines and inflammatory molecules are the crucial factors in MDS which receive more attention now than ever. Altering the microenvironment of the bone marrow strongly affects the cellular components including HSCPs. Since SBSN is a signalling molecule, we can assume that its aberrant expression affects these cells as well. SBSN induced signalling pathways (e.g. Akt, Wnt, or p38), and is associated with therapy resistance, as we showed in previous study.

Recently, the reduced expression of *SBSN* was detected in skin of atopic dermatitis patients. IL-4/IL-13-induced apoptosis of keratinocytes in human living skin equivalents model following *SBSN* knockdown. This suggests that symptoms associated with the condition are promoted via dysregulated Th2 lymphocytes-induced response in human skin (Aoshima et al., 2019). Thus, there is a role for *SBSN* in immune regulation. Furthermore, *SBSN*-null mice lacked Treg generation in the spleen in response to low-dose nickel challenge (Nakazawa et al., 2020). Mechanisms were not clarified, yet this report further supports the role of *SBSN* in immune regulation, which is in accordance with our results from MDS, where *SBSN* expression was mediated by MDSCs, the immune suppressive compartment.

9. Conclusions

To conclude, we showed the observations showing that therapy promotes therapy-resistant phenotype. Besides irradiation, 5-AC induced IFN signalling-dependent therapy-resistant anoikis-resistant phenotype in various cancer cells lines. Additionally, IFNγ treatment induced similar phenotype. Therefore, extrinsic approaches targeting the cancer cells, as well as intrinsic cancer-eradicating mechanisms, such as immune-surveillance, both promote therapy resistance in cancer cells. Understanding underlying mechanisms represent challenges of current cancer therapies.

Among hundreds of differentially expressed genes in therapy-resistant low-adherent cells, we selected oncogene *SBSN* to determine its role in the phenotype. Knockdown of Erk/IFNγ-regulated *SBSN* markedly reduced the abundance of low-adherent cells, indicating that *SBSN* is a crucial mediator of therapy-resistant anoikis-resistant phenotype.

5-AC promotes *SBSN* expression and 5-AC is a first-line therapy in MDS. Indeed, we showed aberrant expression of *SBSN* in the bone marrow of MDS, but this was not mediated by 5-AC therapy. Instead, the expression of *SBSN* was mediated by MDSCs in the bone marrow of a subgroup of MDS patients.

MDSCs are crucial in MDS pathogenesis and progression. Understanding their role in the context of MDS is essential in order to identify novel therapeutic approaches. Identifying efficient treatments for MDS is problematic. This is a result of insufficient stratification which is due to lack of biomarkers (Winter et al., 2020).

Here we identified SBSN as a candidate biomarker for a subgroup of poor-prognosis MDS patients. Elevated SBSN levels marked reduced abundance of T cells and CCL2 levels in the bone marrow. This could indicate a lower potential for immune-based therapies requiring T cells infiltration. Additionally, patients with the highest SBSN levels are likely pre-leukemic, hence a specific approach might be suitable for this group of patients, once supported with a larger cohort of patients.

Altogether, our results indicate that *SBSN* plays a role in therapy resistance and its associated phenotype. *SBSN* is upregulated in therapy-induced therapy-resistant cancer cells, and its aberrant expression in the bone marrow of MDS marks reduced T cells infiltration. Recent observations showed that *SBSN* inhibited T-cell-induced apoptosis and Treg development.

10. Perspectives

There are several emerging questions that need to be addressed in future studies. Importantly, the mechanism of *SBSN*-mediated survival in low-adherent cells requires deeper understanding. SBSN is reported to induce several signalling cascades that were not addressed in our study, such as Wnt or p38 pathway. Thus it would be of interest to elucidate their participation in the phenotype.

Given the receptors of SBSN are not known; certainly these are of great importance. SBSN isoforms represent signalling molecule, thus revealing their receptors would provide hints for future studies deciphering physiological and pathological function of SBSN. Systemic effects of SBSN are likely to occur, especially in human malignancies, hence knowing the receptors, systemic targets could be evaluated as well.

We showed that SBSN represents a candidate biomarker in MDS. In follow up studies we would like to utilize a greater cohort of patients and to follow the changes of SBSN levels over time in individual patients. This is required to provide a comprehensive picture of SBSN level changes and its potential as a biomarker of the disease progression. Certainly, we would like to elucidate whether sudden reduction of SBSN levels in the bone marrow of MDS predicts the onset of the leukemic transformation prior elevation of the blast count. This also requires a prolonged study.

Since SBSN levels showed a significant anti-correlation with T cells and CCL2, we can speculate that patients with the highest SBSN levels might not benefit from immune-based approaches requiring T cell infiltration, such as autologous adoptive T cell transfer, ICI, or DC therapy. Once recruited into clinical trials applying these approaches, SBSN levels could be measured and evaluated together with the trials.

SBSN represents an interesting target for future studies, yet we can assume there are multiple problems to be solved. The posttranslational modifications of SBSN isoforms are elusive. The posttranscriptional regulations of *SBSN* expression are untouched. Mechanisms responsible for aberrant expression of *SBSN* in human malignancies are also undiscovered. To embrace these attempts we compiled all current knowledge of *SBSN* in a yet to be published review. The latest version of the review is attached.

11. Literature

Adam, A.P., George, A., Schewe, D., Bragado, P., Iglesias, B. V., Ranganathan, A.C., Kourtidis, A., Conklin, D.S., and Aguirre-Ghiso, J.A. (2009). Computational Identification of a p38SAPK-Regulated Transcription Factor Network Required for Tumor Cell Quiescence. Cancer Res. *69*, 5664–5672.

Aguirre-Ghiso, J.A. (2007). Models, mechanisms and clinical evidence for cancer dormancy. Nat. Rev. Cancer *7*, 834–846.

Al-Hajj, M., Wicha, M.S., Benito-Hernandez, A., Morrison, S.J., and Clarke, M.F. (2003). Prospective identification of tumorigenic breast cancer cells. Proc. Natl. Acad. Sci. *100*, 3983–3988.

Alam, M.T., Nagao-Kitamoto, H., Ohga, N., Akiyama, K., Maishi, N., Kawamoto, T., Shinohara, N., Taketomi, A., Shindoh, M., Hida, Y., et al. (2014). Suprabasin as a novel tumor endothelial cell marker. Cancer Sci. *105*, 1533–1540.

Aldonza, M.B.D., Ku, J., Hong, J.-Y., Kim, D., Yu, S.J., Lee, M.-S., Prayogo, M.C., Tan, S., Kim, D., Han, J., et al. (2020). Prior acquired resistance to paclitaxel relays diverse EGFR-targeted therapy persistence mechanisms. Sci. Adv. *6*, eaav7416.

Alonso, M.M., Diez-Valle, R., Manterola, L., Rubio, A., Liu, D., Cortes-Santiago, N., Urquiza, L., Jauregi, P., de Munain, A.L., Sampron, N., et al. (2011). Genetic and Epigenetic Modifications of Sox2 Contribute to the Invasive Phenotype of Malignant Gliomas. PLoS One *6*, e26740.

Amaravadi, R.K., Yu, D., Lum, J.J., Bui, T., Christophorou, M.A., Evan, G.I., Thomas-Tikhonenko, A., and Thompson, C.B. (2007). Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. J. Clin. Invest. *117*, 326–336.

Anderson, P., and Dische, S. (1981). Local tumor control and the subsequent incidence of distant metastatic disease. Int. J. Radiat. Oncol. *7*, 1645–1648.

Angell, T.E., Lechner, M.G., Smith, A.M., Martin, S.E., Groshen, S.G., Maceri, D.R., Singer, P.A., and Epstein, A.L. (2016). Circulating Myeloid-Derived Suppressor Cells Predict Differentiated Thyroid Cancer Diagnosis and Extent. Thyroid *26*, 381–389.

Aoshima, M., Phadungsaksawasdi, P., Nakazawa, S., Iwasaki, M., Sakabe, J., Umayahara,

T., Yatagai, T., Ikeya, S., Shimauchi, T., and Tokura, Y. (2019). Decreased expression of suprabasin induces aberrant differentiation and apoptosis of epidermal keratinocytes: Possible role for atopic dermatitis. J. Dermatol. Sci. *95*, 107–112.

Arber, D.A., Orazi, A., Hasserjian, R., Thiele, J., Borowitz, M.J., Le Beau, M.M., Bloomfield, C.D., Cazzola, M., and Vardiman, J.W. (2016). The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood *127*, 2391–2405.

Ascierto, P.A., Ferrucci, P.F., Fisher, R., Del Vecchio, M., Atkinson, V., Schmidt, H., Schachter, J., Queirolo, P., Long, G. V., Di Giacomo, A.M., et al. (2019). Dabrafenib, trametinib and pembrolizumab or placebo in BRAF-mutant melanoma. Nat. Med. *25*, 941–946.

Balz, L.M., Bartkowiak, K., Andreas, A., Pantel, K., Niggemann, B., Zänker, K.S., Brandt, B.H., and Dittmar, T. (2012). The interplay of HER2/HER3/PI3K and EGFR/HER2/PLC - γ 1 signalling in breast cancer cell migration and dissemination. J. Pathol. *227*, 234–244.

Barkan, D., Kleinman, H., Simmons, J.L., Asmussen, H., Kamaraju, A.K., Hoenorhoff, M.J., Liu, Z. -y., Costes, S. V., Cho, E.H., Lockett, S., et al. (2008). Inhibition of Metastatic Outgrowth from Single Dormant Tumor Cells by Targeting the Cytoskeleton. Cancer Res. *68*, 6241–6250.

Barreyro, L., Chlon, T.M., and Starczynowski, D.T. (2018). Chronic immune response dysregulation in MDS pathogenesis. Blood *132*, 1553–1560.

Bartkowiak, K., Wieczorek, M., Buck, F., Harder, S., Moldenhauer, J., Effenberger, K.E., Pantel, K., Peter-Katalinic, J., and Brandt, B.H. (2009). Two-Dimensional Differential Gel Electrophoresis of a Cell Line Derived from a Breast Cancer Micrometastasis Revealed a Stem/Progenitor Cell Protein Profile. J. Proteome Res. *8*, 2004–2014.

Bartkowiak, K., Effenberger, K.E., Harder, S., Andreas, A., Buck, F., Peter-Katalinic, J., Pantel, K., and Brandt, B.H. (2010). Discovery of a Novel Unfolded Protein Response Phenotype of Cancer Stem/Progenitor Cells from the Bone Marrow of Breast Cancer Patients. J. Proteome Res. *9*, 3158–3168.

Bartmann, C., Junker, M., Segerer, S.E., Häusler, S.F., Krockenberger, M., and Kämmerer, U. (2016). CD33 + /HLA-DR neg and CD33 + /HLA-DR +/− Cells: Rare Populations in

the Human Decidua with Characteristics of MDSC. Am. J. Reprod. Immunol. *75*, 539–556.

Basham, T.Y., and Merigan, T.C. (1983). Recombinant interferon-gamma increases HLA-DR synthesis and expression. J. Immunol. *130*, 1492–1494.

Begg, K., and Tavassoli, M. (2020). Inside the hypoxic tumour: reprogramming of the DDR and radioresistance. Cell Death Discov. *6*, 77.

Bellucci, R., Martin, A., Bommarito, D., Wang, K., Hansen, S.H., Freeman, G.J., and Ritz, J. (2015). Interferon-γ-induced activation of JAK1 and JAK2 suppresses tumor cell susceptibility to NK cells through upregulation of PD-L1 expression. Oncoimmunology *4*, e1008824.

Benci, J.L., Xu, B., Qiu, Y., Wu, T.J., Dada, H., Twyman-Saint Victor, C., Cucolo, L., Lee, D.S.M., Pauken, K.E., Huang, A.C., et al. (2016). Tumor Interferon Signaling Regulates a Multigenic Resistance Program to Immune Checkpoint Blockade. Cell *167*, 1540-1554.e12.

Bender, C.M., Pao, M.M., and Jones, P.A. (1998). Inhibition of DNA methylation by 5-aza-2'-deoxycytidine suppresses the growth of human tumor cell lines. Cancer Res. *58*, 95–101.

Beran, M. (2000). Intensive chemotherapy for patients with high-risk myelodysplastic syndrome. Int. J. Hematol. *72*, 139–150.

Bewersdorf, J.P., Ardasheva, A., Podoltsev, N.A., Singh, A., Biancon, G., Halene, S., and Zeidan, A.M. (2019). From clonal hematopoiesis to myeloid leukemia and what happens in between: Will improved understanding lead to new therapeutic and preventive opportunities? Blood Rev. *37*, 100587.

Binsfeld, M., Muller, J., Lamour, V., Veirman, K. De, Raeve, H. De, Bellahcène, A., Valckenborgh, E. Van, Baron, F., Beguin, Y., Caers, J., et al. (2016). Granulocytic myeloid-derived suppressor cells promote angiogenesis in the context of multiple myeloma. Oncotarget *7*, 37931–37943.

Bishop, J.A., Ogawa, T., Chang, X., Illei, P.B., Gabrielson, E., Pai, S.I., and Westra, W.H. (2012). HPV Analysis in Distinguishing Second Primary Tumors From Lung Metastases in

Patients With Head and Neck Squamous Cell Carcinoma. Am. J. Surg. Pathol. *36*, 142–148.

Bosserhoff, A., Kortylewski, M., Komyod, W., Kauffmann, M.-E., Heinrich, P.C., and Behrmann, I. (2004). Interferon-γ-Mediated Growth Regulation of Melanoma Cells: Involvement of STAT1-Dependent and STAT1-Independent Signals. J. Invest. Dermatol. *122*, 414–422.

Bragado, P., Estrada, Y., Parikh, F., Krause, S., Capobianco, C., Farina, H.G., Schewe, D.M., and Aguirre-Ghiso, J.A. (2013). TGF-β2 dictates disseminated tumour cell fate in target organs through TGF-β-RIII and p38α/β signalling. Nat. Cell Biol. *15*, 1351–1361.

Brass, E.P., Peters, M.A., Hinchcliff, K.W., He, Y.D., and Ulrich, R.G. (2009). Temporal pattern of skeletal muscle gene expression following endurance exercise in Alaskan sled dogs. J. Appl. Physiol. *107*, 605–612.

Brastianos, P.K., Carter, S.L., Santagata, S., Cahill, D.P., Taylor-Weiner, A., Jones, R.T., Van Allen, E.M., Lawrence, M.S., Horowitz, P.M., Cibulskis, K., et al. (2015). Genomic Characterization of Brain Metastases Reveals Branched Evolution and Potential Therapeutic Targets. Cancer Discov. *5*, 1164–1177.

Braumüller, H., Wieder, T., Brenner, E., Aßmann, S., Hahn, M., Alkhaled, M., Schilbach, K., Essmann, F., Kneilling, M., Griessinger, C., et al. (2013). T-helper-1-cell cytokines drive cancer into senescence. Nature *494*, 361–365.

Braun, T., and Fenaux, P. (2013). Myelodysplastic Syndromes (MDS) and autoimmune disorders (AD): Cause or consequence? Best Pract. Res. Clin. Haematol. *26*, 327–336.

Bronte, V., Chappell, D.B., Apolloni, E., Cabrelle, A., Wang, M., Hwu, P., and Restifo, N.P. (1999). Unopposed production of granulocyte-macrophage colony-stimulating factor by tumors inhibits CD8+ T cell responses by dysregulating antigen-presenting cell maturation. J. Immunol. *162*, 5728–5737.

Bronte, V., Brandau, S., Chen, S.H., Colombo, M.P., Frey, A.B., Greten, T.F., Mandruzzato, S., Murray, P.J., Ochoa, A., Ostrand-Rosenberg, S., et al. (2016). Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat. Commun. *7*, 1–10.

Carbonell, W.S., Ansorge, O., Sibson, N., and Muschel, R. (2009). The Vascular Basement Membrane as “Soil” in Brain Metastasis. PLoS One *4*, e5857.

Chen, G., Huang, A.C., Zhang, W., Zhang, G., Wu, M., Xu, W., Yu, Z., Yang, J., Wang, B., Sun, H., et al. (2018). Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. Nature *560*, 382–386.

Chen, G., Liu, B., Yin, S., Li, S., Guo, Y., Wang, M., Wang, K., and Wan, X. (2020). Hypoxia induces an endometrial cancer stem-like cell phenotype via HIF-dependent demethylation of SOX2 mRNA. Oncogenesis *9*, 81.

Chen, H.-Y., Shao, C.-J., Chen, F.-R., Kwan, A.-L., and Chen, Z.-P. (2010). Role of ERCC1 promoter hypermethylation in drug resistance to cisplatin in human gliomas. Int. J. Cancer *126*, 1944–1954.

Chen, M.-F., Kuan, F.-C., Yen, T.-C., Lu, M.-S., Lin, P.-Y., Chung, Y.-H., Chen, W.-C., and Lee, K.-D. (2014). IL-6-stimulated CD11b+CD14+HLA-DR− myeloid-derived suppressor cells, are associated with progression and poor prognosis in squamous cell carcinoma of the esophagus. Oncotarget *5*, 8716–8728.

Chen, X., Eksioglu, E.A., Zhou, J., Zhang, L., Djeu, J., Fortenbery, N., Epling-Burnette, P., Van Bijnen, S., Dolstra, H., Cannon, J., et al. (2013). Induction of myelodysplasia by myeloid-derived suppressor cells. J. Clin. Invest. *123*, 4595–4611.

Cheung, K.J., and Ewald, A.J. (2016). A collective route to metastasis: Seeding by tumor cell clusters. Science (80-.). *352*, 167–169.

Cheung, K.J., Padmanaban, V., Silvestri, V., Schipper, K., Cohen, J.D., Fairchild, A.N., Gorin, M.A., Verdone, J.E., Pienta, K.J., Bader, J.S., et al. (2016). Polyclonal breast cancer metastases arise from collective dissemination of keratin 14-expressing tumor cell clusters. Proc. Natl. Acad. Sci. *113*, E854–E863.

Chiappinelli, K.B., Strissel, P.L., Desrichard, A., Li, H., Henke, C., Akman, B., Hein, A., Rote, N.S., Cope, L.M., Snyder, A., et al. (2015). Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses. Cell *162*, 974–986.

Chiappinelli, K.B., Zahnow, C.A., Ahuja, N., and Baylin, S.B. (2016). Combining

Epigenetic and Immunotherapy to Combat Cancer. Cancer Res. *76*, 1683–1689.

Christman, J.K. (2002). 5-Azacytidine and 5-aza-2′-deoxycytidine as inhibitors of DNA methylation: Mechanistic studies and their implications for cancer therapy. Oncogene *21*, 5483–5495.

Coates, P.J., Lorimore, S.A., and Wright, E.G. (2005). Cell and tissue responses to genotoxic stress. J. Pathol. *205*, 221–235.

Condamine, T., Mastio, J., and Gabrilovich, D.I. (2015). Transcriptional regulation of myeloid-derived suppressor cells. J. Leukoc. Biol. *98*, 913–922.

Corzo, C.A., Cotter, M.J., Cheng, P., Cheng, F., Kusmartsev, S., Sotomayor, E., Padhya, T., McCaffrey, T. V., McCaffrey, J.C., and Gabrilovich, D.I. (2009). Mechanism Regulating Reactive Oxygen Species in Tumor-Induced Myeloid-Derived Suppressor Cells. J. Immunol. *182*, 5693–5701.

Costello, J.F., Frühwald, M.C., Smiraglia, D.J., Rush, L.J., Robertson, G.P., Gao, X., Wright, F.A., Feramisco, J.D., Peltomäki, P., Lang, J.C., et al. (2000). Aberrant CpG-island methylation has non-random and tumour-type–specific patterns. Nat. Genet. *24*, 132–138.

Coussens, L.M., and Werb, Z. (2002). Inflammation and cancer. Nature *420*, 860–867.

Covey, J.M., and Zaharko, D.S. (1985). Comparison of the in vitro cytotoxicity (L1210) of 5-Aza-2′-deoxycytidine with its therapeutic and toxic effects in mice. Eur. J. Cancer Clin. Oncol. *21*, 109–117.

Cummins, E.P., Keogh, C.E., Crean, D., and Taylor, C.T. (2016). The role of HIF in immunity and inflammation. Mol. Aspects Med. *47–48*, 24–34.

Dal Pra, A., Cury, F.L., and Souhami, L. (2010). Combining radiation therapy and androgen deprivation for localized prostate cancer—a critical review. Curr. Oncol. *17*, 28–38.

Dasari, S., and Bernard Tchounwou, P. (2014). Cisplatin in cancer therapy: Molecular mechanisms of action. Eur. J. Pharmacol. *740*, 364–378.

Daver, N., Boddu, P., Garcia-Manero, G., Yadav, S.S., Sharma, P., Allison, J., and

Kantarjian, H. (2018). Hypomethylating agents in combination with immune checkpoint inhibitors in acute myeloid leukemia and myelodysplastic syndromes. Leukemia *32*, 1094–1105.

Demaria, M., O'Leary, M.N., Chang, J., Shao, L., Liu, S., Alimirah, F., Koenig, K., Le, C., Mitin, N., Deal, A.M., et al. (2017). Cellular Senescence Promotes Adverse Effects of Chemotherapy and Cancer Relapse. Cancer Discov. *7*, 165–176.

Diaz-Montero, C.M.M., Salem, M.L.L., Nishimura, M.I.I., Garrett-Mayer, E., Cole, D.J.J., and Montero, A.J.J. (2009). Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin–cyclophosphamide chemotherapy. Cancer Immunol. Immunother. *58*, 49–59.

Dolcetti, L., Peranzoni, E., Ugel, S., Marigo, I., Fernandez Gomez, A., Mesa, C., Geilich, M., Winkels, G., Traggiai, E., Casati, A., et al. (2009). Hierarchy of immunosuppressive strength among myeloid-derived suppressor cell subsets is determined by GM-CSF. Eur. J. Immunol. *40*, 22–35.

Dongre, A., and Weinberg, R.A. (2019). New insights into the mechanisms of epithelial–mesenchymal transition and implications for cancer. Nat. Rev. Mol. Cell Biol. *20*, 69–84.

Durante, M., and Loeffler, J.S. (2010). Charged particles in radiation oncology. Nat. Rev. Clin. Oncol. *7*, 37–43.

Ebbing, E.A., van der Zalm, A.P., Steins, A., Creemers, A., Hermsen, S., Rentenaar, R., Klein, M., Waasdorp, C., Hooijer, G.K.J., Meijer, S.L., et al. (2019). Stromal-derived interleukin 6 drives epithelial-to-mesenchymal transition and therapy resistance in esophageal adenocarcinoma. Proc. Natl. Acad. Sci. *116*, 2237–2242.

Ebelt, N.D., Zuniga, E., Johnson, B.L., Diamond, D.J., and Manuel, E.R. (2020). 5-Azacytidine Potentiates Anti-tumor Immunity in a Model of Pancreatic Ductal Adenocarcinoma. Front. Immunol. *11*, 538.

Ebert, P.J.R., Cheung, J., Yang, Y., McNamara, E., Hong, R., Moskalenko, M., Gould, S.E., Maecker, H., Irving, B.A., Kim, J.M., et al. (2016). MAP Kinase Inhibition Promotes T Cell and Anti-tumor Activity in Combination with PD-L1 Checkpoint Blockade. Immunity *44*, 609–621.

Eriksson, D., and Stigbrand, T. (2010). Radiation-induced cell death mechanisms. Tumor Biol. *31*, 363–372.

von Essen, C.F. (1991). Radiation enhancement of metastasis: a review. Clin. Exp. Metastasis *9*, 77–104.

Farrar, J.D., Katz, K.H., Windsor, J., Thrush, G., Scheuermann, R.H., Uhr, J.W., and Street, N.E. (1999). Cancer dormancy. VII. A regulatory role for CD8+ T cells and IFN-gamma in establishing and maintaining the tumor-dormant state. J. Immunol. *162*, 2842–2849.

Ferlay, J., Colombet, M., Soerjomataram, I., Mathers, C., Parkin, D.M., Piñeros, M., Znaor, A., and Bray, F. (2019). Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int. J. Cancer *144*, 1941–1953.

Festuccia, M., Baker, K., Gooley, T.A., Sandmaier, B.M., Deeg, H.J., and Scott, B.L. (2017). Hematopoietic Cell Transplantation in Myelodysplastic Syndromes after Treatment with Hypomethylating Agents. Biol. Blood Marrow Transplant. *23*, 1509–1514.

Fink, D., Zheng, H., Nebel, S., Norris, P.S., Aebi, S., Lin, T.P., Nehmé, A., Christen, R.D., Haas, M., MacLeod, C.L., et al. (1997). In vitro and in vivo resistance to cisplatin in cells that have lost DNA mismatch repair. Cancer Res. *57*, 1841–1845.

Formolo, C.A., Williams, R., Gordish-Dressman, H., MacDonald, T.J., Lee, N.H., and Hathout, Y. (2011). Secretome signature of invasive glioblastoma multiforme. J. Proteome Res. *10*, 3149–3159.

Francart, M.-E., Lambert, J., Vanwynsberghe, A.M., Thompson, E.W., Bourcy, M., Polette, M., and Gilles, C. (2018). Epithelial-mesenchymal plasticity and circulating tumor cells: Travel companions to metastases. Dev. Dyn. *247*, 432–450.

Frederick, D.T., Piris, A., Cogdill, A.P., Cooper, Z.A., Lezcano, C., Ferrone, C.R., Mitra, D., Boni, A., Newton, L.P., Liu, C., et al. (2013). BRAF Inhibition Is Associated with Enhanced Melanoma Antigen Expression and a More Favorable Tumor Microenvironment in Patients with Metastatic Melanoma. Clin. Cancer Res. *19*, 1225–1231.

Fridman, J.S., and Lowe, S.W. (2003). Control of apoptosis by p53. Oncogene *22*, 9030–9040.

Friedman, H., Newton, C., and Klein, T.W. (2003). Microbial Infections, Immunomodulation, and Drugs of Abuse. Clin. Microbiol. Rev. *16*, 209–219.

Gabrilovich, D.I., Ostrand-Rosenberg, S., and Bronte, V. (2012). Coordinated regulation of myeloid cells by tumours. Nat. Rev. Immunol. *12*, 253–268.

Gao, X.L., Zhang, M., Tang, Y.L., and Liang, X.H. (2017). Cancer cell dormancy: mechanisms and implications of cancer recurrence and metastasis. Onco. Targets. Ther. *Volume 10*, 5219–5228.

Garcia-Diaz, A., Shin, D.S., Moreno, B.H., Saco, J., Escuin-Ordinas, H., Rodriguez, G.A., Zaretsky, J.M., Sun, L., Hugo, W., Wang, X., et al. (2017). Interferon Receptor Signaling Pathways Regulating PD-L1 and PD-L2 Expression. Cell Rep. *19*, 1189–1201.

Garcia-Manero, G., Daver, N.G., Montalban-Bravo, G., Jabbour, E.J., DiNardo, C.D., Kornblau, S.M., Bose, P., Alvarado, Y., Ohanian, M., Borthakur, G., et al. (2016). A Phase II Study Evaluating the Combination of Nivolumab (Nivo) or Ipilimumab (Ipi) with Azacitidine in Pts with Previously Treated or Untreated Myelodysplastic Syndromes (MDS). Blood *128*, 344–344.

Gaykalova, D., Vatapalli, R., Glazer, C.A., Bhan, S., Shao, C., Sidransky, D., Ha, P.K., and Califano, J.A. (2012). Dose-Dependent Activation of Putative Oncogene SBSN by BORIS. PLoS One *7*, e40389.

Ghajar, C.M., Peinado, H., Mori, H., Matei, I.R., Evason, K.J., Brazier, H., Almeida, D., Koller, A., Hajjar, K.A., Stainier, D.Y.R., et al. (2013). The perivascular niche regulates breast tumour dormancy. Nat. Cell Biol. *15*, 807–817.

Ghobrial, I.M., Detappe, A., Anderson, K.C., and Steensma, D.P. (2018). The bone-marrow niche in MDS and MGUS: Implications for AML and MM. Nat. Rev. Clin. Oncol. *15*, 219–233.

Giannoni, E., Bianchini, F., Masieri, L., Serni, S., Torre, E., Calorini, L., and Chiarugi, P. (2010). Reciprocal Activation of Prostate Cancer Cells and Cancer-Associated Fibroblasts Stimulates Epithelial-Mesenchymal Transition and Cancer Stemness. Cancer Res. *70*, 6945–6956.

Gigerenzer, G., and Wegwarth, O. (2013). Five year survival rates can mislead. BMJ *346*,

f548–f548.

Gilbert, M.R., Wang, M., Aldape, K.D., Stupp, R., Hegi, M.E., Jaeckle, K.A., Armstrong, T.S., Wefel, J.S., Won, M., Blumenthal, D.T., et al. (2013). Dose-Dense Temozolomide for Newly Diagnosed Glioblastoma: A Randomized Phase III Clinical Trial. J. Clin. Oncol. *31*, 4085–4091.

Glazer, C.A., Smith, I.M., Ochs, M.F., Begum, S., Westra, W., Chang, S.S., Sun, W., Bhan, S., Khan, Z., Ahrendt, S., et al. (2009). Integrative Discovery of Epigenetically Derepressed Cancer Testis Antigens in NSCLC. PLoS One *4*, e8189.

Goddard, E.T., Bozic, I., Riddell, S.R., and Ghajar, C.M. (2018). Dormant tumour cells, their niches and the influence of immunity. Nat. Cell Biol. *20*, 1240–1249.

Goyal, R., and Longo, L.D. (2014). Acclimatization to long-term hypoxia: gene expression in ovine carotid arteries. Physiol Genomics *46*, 725–734.

Grasso, C.S., Giannakis, M., Wells, D.K., Hamada, T., Mu, X.J., Quist, M., Nowak, J.A., Nishihara, R., Qian, Z.R., Inamura, K., et al. (2018). Genetic Mechanisms of Immune Evasion in Colorectal Cancer. Cancer Discov. *8*, 730–749.

Greenberg, P., Cox, C., LeBeau, M.M., Fenaux, P., Morel, P., Sanz, G., Sanz, M., Vallespi, T., Hamblin, T., Oscier, D., et al. (1997a). International Scoring System for Evaluating Prognosis in Myelodysplastic Syndromes. Blood *89*, 2079–2088.

Greenberg, P., Cox, C., LeBeau, M.M., Fenaux, P., Morel, P., Sanz, G., Sanz, M., Vallespi, T., Hamblin, T., Oscier, D., et al. (1997b). International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood *89*, 2079–2088.

Greenberg, P.L., Tuechler, H., Schanz, J., Sanz, G., Garcia-Manero, G., Solé, F., Bennett, J.M., Bowen, D., Fenaux, P., Dreyfus, F., et al. (2012). Revised International Prognostic Scoring System for Myelodysplastic Syndromes. Blood *120*, 2454–2465.

Greten, F.R., and Grivennikov, S.I. (2019). Inflammation and Cancer: Triggers, Mechanisms, and Consequences. Immunity *51*, 27–41.

Grivennikov, S.I., Greten, F.R., and Karin, M. (2010). Immunity, Inflammation, and Cancer. Cell *140*, 883–899.

Guida, M., Maraldi, T., Beretti, F., Follo, M.Y., Manzoli, L., and De Pol, A. (2014). Nuclear Nox4-Derived Reactive Oxygen Species in Myelodysplastic Syndromes. Biomed Res. Int. *2014*, 1–11.

Haferlach, T., Nagata, Y., Grossmann, V., Okuno, Y., Bacher, U., Nagae, G., Schnittger, S., Sanada, M., Kon, A., Alpermann, T., et al. (2014). Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia *28*, 241–247.

Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of Cancer: The Next Generation. Cell *144*, 646–674.

Hanson, E.M., Clements, V.K., Sinha, P., Ilkovitch, D., and Ostrand-Rosenberg, S. (2009). Myeloid-Derived Suppressor Cells Down-Regulate L-Selectin Expression on CD4 + and CD8 + T Cells. J. Immunol. *183*, 937–944.

Hasserjian, R.P. (2019). Myelodysplastic Syndrome Updated. Pathobiology *86*, 53–61.

Herrscher, H., and Robert, C. (2020). Immune checkpoint inhibitors in melanoma in the metastatic, neoadjuvant, and adjuvant setting. Curr. Opin. Oncol. *32*, 106–113.

Holmgren, L., O'Reilly, M.S., and Folkman, J. (1995). Dormancy of micrometastases: Balanced proliferation and apoptosis in the presence of angiogenesis suppression. Nat. Med. *1*, 149–153.

Holohan, C., Van Schaeybroeck, S., Longley, D.B., and Johnston, P.G. (2013). Cancer drug resistance: An evolving paradigm. Nat. Rev. Cancer *13*, 714–726.

Höpken, U.E., and Rehm, A. (2019). Targeting the Tumor Microenvironment of Leukemia and Lymphoma. Trends in Cancer *5*, 351–364.

Hoshino, A., Costa-Silva, B., Shen, T.-L., Rodrigues, G., Hashimoto, A., Tesic Mark, M., Molina, H., Kohsaka, S., Di Giannatale, A., Ceder, S., et al. (2015). Tumour exosome integrins determine organotropic metastasis. Nature *527*, 329–335.

Housman, G., Byler, S., Heerboth, S., Lapinska, K., Longacre, M., Snyder, N., and Sarkar, S. (2014). Drug resistance in cancer: an overview. Cancers (Basel). *6*, 1769–1792.

Howard, J.H., Frolov, A., Tzeng, C.-W.D., Stewart, A., Midzak, A., Majmundar, A., Godwin, A., Heslin, M., Heslin, M., Bellacosa, A., et al. (2009). Epigenetic

downregulation of the DNA repair gene MED1/MBD4 in colorectal and ovarian cancer. Cancer Biol. Ther. *8*, 94–100.

Huang, Z., Su, W., Lu, T., Wang, Y., Dong, Y., Qin, Y., Liu, D., Sun, L., and Jiao, W. (2020). First-Line Immune-Checkpoint Inhibitors in Non-Small Cell Lung Cancer: Current Landscape and Future Progress. Front. Pharmacol. *11*, 578091.

Hubackova, S., Kucerova, A., Michlits, G., Kyjacova, L., Reinis, M., Korolov, O., Bartek, J., and Hodny, Z. (2015). IFNγ induces oxidative stress, DNA damage and tumor cell senescence via TGFβ/SMAD signaling-dependent induction of Nox4 and suppression of ANT2. Oncogene *35*, 1–14.

Hubackova, S., Pribyl, M., Kyjacova, L., Moudra, A., Dzijak, R., Salovska, B., Strnad, H., Tambor, V., Imrichova, T., Svec, J., et al. (2019). Interferon-regulated suprabasin is essential for stress-induced stem-like cell conversion and therapy resistance of human malignancies. Mol. Oncol. *13*, 1467–1489.

Humphries, C. (2013). Adoptive cell therapy: Honing that killer instinct. Nature *504*, S13–S15.

Hunt, S.E., McLaren, W., Gil, L., Thormann, A., Schuilenburg, H., Sheppard, D., Parton, A., Armean, I.M., Trevanion, S.J., Flicek, P., et al. (2018). Ensembl variation resources. Database *2018*.

Hüsemann, Y., Geigl, J.B., Schubert, F., Musiani, P., Meyer, M., Burghart, E., Forni, G., Eils, R., Fehm, T., Riethmüller, G., et al. (2008). Systemic Spread Is an Early Step in Breast Cancer. Cancer Cell *13*, 58–68.

Ichinose, K., Ohyama, K., Furukawa, K., Higuchi, O., Mukaino, A., Satoh, K., Nakane, S., Shimizu, T., Umeda, M., Fukui, S., et al. (2018). Novel anti-suprabasin antibodies may contribute to the pathogenesis of neuropsychiatric systemic lupus erythematosus. Clin. Immunol. *193*, 123–130.

Iwata, T., Kondo, Y., Kimura, O., Morosawa, T., Fujisaka, Y., Umetsu, T., Kogure, T., Inoue, J., Nakagome, Y., and Shimosegawa, T. (2016). PD-L1+MDSCs are increased in HCC patients and induced by soluble factor in the tumor microenvironment. Sci. Rep. *6*, 39296.

Jaiswal, S., and Libby, P. (2020). Clonal haematopoiesis: connecting ageing and inflammation in cardiovascular disease. Nat. Rev. Cardiol. *17*, 137–144.

Jaiswal, B.S., Durinck, S., Stawiski, E.W., Yin, J., Wang, W., Lin, E., Moffat, J., Martin, S.E., Modrusan, Z., and Seshagiri, S. (2018). ERK Mutations and Amplification Confer Resistance to ERK-Inhibitor Therapy. Clin. Cancer Res. *24*, 4044–4055.

James, S.R., Link, P.A., and Karpf, A.R. (2006). Epigenetic regulation of X-linked cancer/germline antigen genes by DNMT1 and DNMT3b. Oncogene *25*, 6975–6985.

Jiménez-Sánchez, A., Memon, D., Pourpe, S., Veeraraghavan, H., Li, Y., Vargas, H.A., Gill, M.B., Park, K.J., Zivanovic, O., Konner, J., et al. (2017). Heterogeneous Tumor-Immune Microenvironments among Differentially Growing Metastases in an Ovarian Cancer Patient. Cell *170*, 927-938.e20.

Jimsheleishvili, S., Alshareef, A.T., Papadimitriou, K., Bregy, A., Shah, A.H., Graham, R.M., Ferraro, N., and Komotar, R.J. (2014). Extracranial glioblastoma in transplant recipients. J. Cancer Res. Clin. Oncol. *140*, 801–807.

Jing, Y., Han, Z., Zhang, S., Liu, Y., and Wei, L. (2011). Epithelial-Mesenchymal Transition in tumor microenvironment. Cell Biosci. *1*, 29.

Johnson, B., Achyut, B., Fulzele, S., Mondal, A., Kolhe, R., and Arbab, A. (2018). Delineating Pro-Angiogenic Myeloid Cells in Cancer Therapy. Int. J. Mol. Sci. *19*, 2565.

Jordan, K.R.R., Amaria, R.N.N., Ramirez, O., Callihan, E.B.B., Gao, D., Borakove, M., Manthey, E., Borges, V.F.F., and McCarter, M.D.D. (2013). Myeloid-derived suppressor cells are associated with disease progression and decreased overall survival in advanced-stage melanoma patients. Cancer Immunol. Immunother. *62*, 1711–1722.

June, C.H., O'Connor, R.S., Kawalekar, O.U., Ghassemi, S., and Milone, M.C. (2018). CAR T cell immunotherapy for human cancer. Science (80-.). *359*, 1361–1365.

Juttermann, R., Li, E., and Jaenisch, R. (1994). Toxicity of 5-aza-2'-deoxycytidine to mammalian cells is mediated primarily by covalent trapping of DNA methyltransferase rather than DNA demethylation. Proc. Natl. Acad. Sci. *91*, 11797–11801.

Kalbasi, A., and Ribas, A. (2020). Tumour-intrinsic resistance to immune checkpoint blockade. Nat. Rev. Immunol. *20*, 25–39.

Kale, J., Osterlund, E.J., and Andrews, D.W. (2018). BCL-2 family proteins: changing partners in the dance towards death. Cell Death Differ. *25*, 65–80.

Kaminskas, E. (2005). Approval Summary: Azacitidine for Treatment of Myelodysplastic Syndrome Subtypes. Clin. Cancer Res. *11*, 3604–3608.

Kang, X., Zhang, X., Liu, Z., Xu, H., Wang, T., He, L., and Zhao, A. (2016). Granulocytic myeloid-derived suppressor cells maintain feto-maternal tolerance by inducing Foxp3 expression in CD4 + CD25 − T cells by activation of the TGF-β/β-catenin pathway. Mol. Hum. Reprod. *22*, 499–511.

Kaplan, R.N., Riba, R.D., Zacharoulis, S., Bramley, A.H., Vincent, L., Costa, C., MacDonald, D.D., Jin, D.K., Shido, K., Kerns, S.A., et al. (2005). VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature *438*, 820–827.

Karpf, A.R. (2006). A Potential Role for Epigenetic Modulatory Drugs in the Enhancement of Cancer/Germ-Line Antigen Vaccine Efficacy. Epigenetics *1*, 116–120.

Karpf, A.R., Peterson, P.W., Rawlins, J.T., Dalley, B.K., Yang, Q., Albertsen, H., and Jones, D.A. (1999). Inhibition of DNA methyltransferase stimulates the expression of signal transducer and activator of transcription 1, 2, and 3 genes in colon tumor cells. Proc. Natl. Acad. Sci. *96*, 14007–14012.

Karpf, A.R., Moore, B.C., Ririe, T.O., and Jones, D.A. (2001). Activation of the p53 DNA damage response pathway after inhibition of DNA methyltransferase by 5-aza-2'-deoxycytidine. Mol. Pharmacol. *59*, 751–757.

Karpf, A.R., Lasek, A.W., Ririe, T.O., Hanks, A.N., Grossman, D., and Jones, D.A. (2004). Limited Gene Activation in Tumor and Normal Epithelial Cells Treated with the DNA Methyltransferase Inhibitor 5-Aza-2′-deoxycytidine. Mol. Pharmacol. *65*, 18–27.

Kiecolt-Glaser, J.K., and Glaser, R. (1995). Psychoneuroimmunology and Health Consequences. Psychosom. Med. *57*, 269–274.

Kim, J., Kong, J., Chang, H., Kim, H., and Kim, A. (2016). EGF induces epithelial-mesenchymal transition through phospho-Smad2/3-Snail signaling pathway in breast cancer cells. Oncotarget *7*, 85021–85032.

Klein, C.A. (2011). Framework models of tumor dormancy from patient-derived observations. Curr. Opin. Genet. Dev. *21*, 42–49.

Kobayashi, A., Okuda, H., Xing, F., Pandey, P.R., Watabe, M., Hirota, S., Pai, S.K., Liu, W., Fukuda, K., Chambers, C., et al. (2011). Bone morphogenetic protein 7 in dormancy and metastasis of prostate cancer stem-like cells in bone. J. Exp. Med. *208*, 2641–2655.

Konopleva, M., Contractor, R., Tsao, T., Samudio, I., Ruvolo, P.P., Kitada, S., Deng, X., Zhai, D., Shi, Y.-X., Sneed, T., et al. (2006). Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. Cancer Cell *10*, 375–388.

Koźmiński, P., Halik, P.K., Chesori, R., and Gniazdowska, E. (2020). Overview of Dual-Acting Drug Methotrexate in Different Neurological Diseases, Autoimmune Pathologies and Cancers. Int. J. Mol. Sci. *21*, 3483.

Krönke, J., Fink, E.C., Hollenbach, P.W., MacBeth, K.J., Hurst, S.N., Udeshi, N.D., Chamberlain, P.P., Mani, D.R., Man, H.W., Gandhi, A.K., et al. (2015). Lenalidomide induces ubiquitination and degradation of CK1α in del(5q) MDS. Nature *523*, 183–188.

Kyjacova, L., Hubackova, S., Krejcikova, K., Strauss, R., Hanzlikova, H., Dzijak, R., Imrichova, T., Simova, J., Reinis, M., Bartek, J., et al. (2014). Radiotherapy-induced plasticity of prostate cancer mobilizes stem-like non-adherent, Erk signaling-dependent cells. Cell Death Differ. *22*, 1–14.

Lämmermann, T., and Sixt, M. (2009). Mechanical modes of “amoeboid” cell migration. Curr. Opin. Cell Biol. *21*, 636–644.

Ledermann, J.A. (2018). First-line treatment of ovarian cancer: questions and controversies to address. Ther. Adv. Med. Oncol. *10*, 175883591876823.

Lee, S.S.-E., Lim, J.-Y., Ryu, D.-B., Kim, T.W.W., Yoon, J.-H., Cho, B.-S., Eom, K.-S., Kim, Y.-J., Kim, H.-J., Lee, S.S.-E., et al. (2016). Circulating immune cell phenotype can predict the outcome of lenalidomide plus low-dose dexamethasone treatment in patients with refractory/relapsed multiple myeloma. Cancer Immunol. Immunother. *65*, 983–994.

Lehmann, S., te Boekhorst, V., Odenthal, J., Bianchi, R., van Helvert, S., Ikenberg, K., Ilina, O., Stoma, S., Xandry, J., Jiang, L., et al. (2017). Hypoxia Induces a HIF-1-

Dependent Transition from Collective-to-Amoeboid Dissemination in Epithelial Cancer Cells. Curr. Biol. *27*, 392–400.

Liang, Y., Tebaldi, T., Rejeski, K., Joshi, P., Stefani, G., Taylor, A., Song, Y., Vasic, R., Maziarz, J., Balasubramanian, K., et al. (2018). SRSF2 mutations drive oncogenesis by activating a global program of aberrant alternative splicing in hematopoietic cells. Leukemia *32*, 2659–2671.

Liu, B., Huang, G., Zhu, H., Ma, Z., Tian, X., Yin, L., Gao, X., and He, X. (2019). Analysis of gene co-expression network reveals prognostic significance of CNFN in patients with head and neck cancer. Oncol. Rep. *41*, 2168–2180.

Liu, S., Zhou, X., Peng, X., Li, M., Ren, B., Cheng, G., and Cheng, L. (2020). Porphyromonas gingivalis Promotes Immunoevasion of Oral Cancer by Protecting Cancer from Macrophage Attack. J. Immunol. *205*, 282–289.

Liu, Y., Liang, X., Yin, X., Lv, J., Tang, K., Ma, J., Ji, T., Zhang, H., Dong, W., Jin, X., et al. (2017). Blockade of IDO-kynurenine-AhR metabolic circuitry abrogates IFN-γ-induced immunologic dormancy of tumor-repopulating cells. Nat. Commun. *8*, 15207.

Lu, X., Mu, E., Wei, Y., Riethdorf, S., Yang, Q., Yuan, M., Yan, J., Hua, Y., Tiede, B.J., Lu, X., et al. (2011). VCAM-1 Promotes Osteolytic Expansion of Indolent Bone Micrometastasis of Breast Cancer by Engaging α4β1-Positive Osteoclast Progenitors. Cancer Cell *20*, 701–714.

Lu, Y., Liu, B., Liu, Y., Yu, X., and Cheng, G. (2020). Dual effects of active ERK in cancer: A potential target for enhancing radiosensitivity (Review). Oncol. Lett. *20*, 993–1000.

Lu, Z., Luo, R.Z., Lu, Y., Zhang, X., Yu, Q., Khare, S., Kondo, S., Kondo, Y., Yu, Y., Mills, G.B., et al. (2008). The tumor suppressor gene ARHI regulates autophagy and tumor dormancy in human ovarian cancer cells. J. Clin. Invest. *118*, 3917–3929.

MacKie, R.M., Reid, R., and Junor, B. (2003). Fatal Melanoma Transferred in a Donated Kidney 16 Years after Melanoma Surgery. N. Engl. J. Med. *348*, 567–568.

Magnon, C., Hall, S.J., Lin, J., Xue, X., Gerber, L., Freedland, S.J., and Frenette, P.S. (2013). Autonomic Nerve Development Contributes to Prostate Cancer Progression.

Science (80-.). *341*, 1236361–1236361.

Mahnke, Y.D., Schwendemann, J., Beckhove, P., and Schirrmacher, V. (2005). Maintenance of long-term tumour-specific T-cell memory by residual dormant tumour cells. Immunology *115*, 325–336.

Malanchi, I., Santamaria-Martínez, A., Susanto, E., Peng, H., Lehr, H.-A., Delaloye, J.-F., and Huelsken, J. (2012). Interactions between cancer stem cells and their niche govern metastatic colonization. Nature *481*, 85–89.

Marini, O., Spina, C., Mimiola, E., Cassaro, A., Malerba, G., Todeschini, G., Perbellini, O., Scupoli, M., Carli, G., Facchinelli, D., et al. (2016). Identification of granulocytic myeloid-derived suppressor cells (G-MDSCs) in the peripheral blood of Hodgkin and non-Hodgkin lymphoma patients. Oncotarget *7*, 27676–27688.

Marshall, J.-C.A., Collins, J.W., Nakayama, J., Horak, C.E., Liewehr, D.J., Steinberg, S.M., Albaugh, M., Vidal-Vanaclocha, F., Palmieri, D., Barbier, M., et al. (2012). Effect of Inhibition of the Lysophosphatidic Acid Receptor 1 on Metastasis and Metastatic Dormancy in Breast Cancer. JNCI J. Natl. Cancer Inst. *104*, 1306–1319.

Martin, O.A., Anderson, R.L., Russell, P.A., Ashley Cox, R., Ivashkevich, A., Swierczak, A., Doherty, J.P., Jacobs, D.H.M., Smith, J., Siva, S., et al. (2014). Mobilization of Viable Tumor Cells Into the Circulation During Radiation Therapy. Int. J. Radiat. Oncol. *88*, 395–403.

Masiero, M., Minuzzo, S., Pusceddu, I., Moserle, L., Persano, L., Agnusdei, V., Tosello, V., Basso, G., Amadori, A., and Indraccolo, S. (2011). Notch3-mediated regulation of MKP-1 levels promotes survival of T acute lymphoblastic leukemia cells. Leukemia *25*, 588–598.

Matsui, T., Hayashi-Kisumi, F., Kinoshita, Y., Katahira, S., Morita, K., Miyachi, Y., Ono, Y., Imai, T., Tanigawa, Y., Komiya, T., et al. (2004). Identification of novel keratinocyte-secreted peptides dermokine-α/- β and a new stratified epithelium-secreted protein gene complex on human chromosome 19q13.1. Genomics *84*, 384–397.

Medzhitov, R. (2008). Origin and physiological roles of inflammation. Nature *454*, 428–435.

Molon, B., Ugel, S., Del Pozzo, F., Soldani, C., Zilio, S., Avella, D., De Palma, A., Mauri, P., Monegal, A., Rescigno, M., et al. (2011). Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. J. Exp. Med. *208*, 1949–1962.

Moncharmont, C., Levy, A., Guy, J.B., Falk, A.T., Guilbert, M., Trone, J.C., Alphonse, G., Gilormini, M., Ardail, D., Toillon, R.A., et al. (2014). Radiation-enhanced cell migration/invasion process: A review. Crit. Rev. Oncol. Hematol. *92*, 133–142.

Moudra, A., Hubackova, S., Machalova, V., Vancurova, M., Bartek, J., Reinis, M., Hodny, Z., and Jonasova, A. (2016). Dynamic alterations of bone marrow cytokine landscape of myelodysplastic syndromes patients treated with 5-azacytidine. Oncoimmunology *5*, 1–12.

Mui, U., Haley, C., and Tyring, S. (2017). Viral Oncology: Molecular Biology and Pathogenesis. J. Clin. Med. *6*, 111.

Müller-Hermelink, N., Braumüller, H., Pichler, B., Wieder, T., Mailhammer, R., Schaak, K., Ghoreschi, K., Yazdi, A., Haubner, R., Sander, C.A., et al. (2008). TNFR1 Signaling and IFN-γ Signaling Determine whether T Cells Induce Tumor Dormancy or Promote Multistage Carcinogenesis. Cancer Cell *13*, 507–518.

Muto, T., Walker, C.S., Choi, K., Hueneman, K., Smith, M.A., Gul, Z., Garcia-Manero, G., Ma, A., Zheng, Y., and Starczynowski, D.T. (2020). Adaptive response to inflammation contributes to sustained myelopoiesis and confers a competitive advantage in myelodysplastic syndrome HSCs. Nat. Immunol. *21*, 535–545.

Nagaraj, S., Gupta, K., Pisarev, V., Kinarsky, L., Sherman, S., Kang, L., Herber, D.L., Schneck, J., and Gabrilovich, D.I. (2007). Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer. Nat. Med. *13*, 828–835.

Nair, R.R., Sinha, P., Khanna, A., and Singh, K. (2015). Reduced Myeloid-derived Suppressor Cells in the Blood and Endometrium is Associated with Early Miscarriage. Am. J. Reprod. Immunol. *73*, 479–486.

Nakamura, K., and Smyth, M.J. (2020). Myeloid immunosuppression and immune checkpoints in the tumor microenvironment. Cell. Mol. Immunol. *17*, 1–12.

Nakazawa, S., Shimauchi, T., Funakoshi, A., Aoshima, M., Phadungsaksawasdi, P., Sakabe, J., Asakawa, S., Hirasawa, N., Ito, T., and Tokura, Y. (2020). Suprabasin-null

mice retain skin barrier function and show high contact hypersensitivity to nickel upon oral nickel loading. Sci. Rep. *10*, 14559.

Natsuizaka, M., Whelan, K.A., Kagawa, S., Tanaka, K., Giroux, V., Chandramouleeswaran, P.M., Long, A., Sahu, V., Darling, D.S., Que, J., et al. (2017). Interplay between Notch1 and Notch3 promotes EMT and tumor initiation in squamous cell carcinoma. Nat. Commun. *8*, 1758.

Naume, B., Synnestvedt, M., Falk, R.S., Wiedswang, G., Weyde, K., Risberg, T., Kersten, C., Mjaaland, I., Vindi, L., Sommer, H.H., et al. (2014). Clinical Outcome With Correlation to Disseminated Tumor Cell (DTC) Status After DTC-Guided Secondary Adjuvant Treatment With Docetaxel in Early Breast Cancer. J. Clin. Oncol. *32*, 3848–3857.

Nazio, F., Bordi, M., Cianfanelli, V., Locatelli, F., and Cecconi, F. (2019). Autophagy and cancer stem cells: molecular mechanisms and therapeutic applications. Cell Death Differ. *26*, 690–702.

O'Leary, A. (1990). Stress, emotion, and human immune function. Psychol. Bull. *108*, 363–382.

Oran, B., and Weisdorf, D.J. (2012). Survival for older patients with acute myeloid leukemia: a population-based study. Haematologica *97*, 1916–1924.

Ostrand-Rosenberg, S., and Fenselau, C. (2018). Myeloid-Derived Suppressor Cells: Immune-Suppressive Cells That Impair Antitumor Immunity and Are Sculpted by Their Environment. J. Immunol. *200*, 422–431.

Øyan, A.M., Bø, T.H., Jonassen, I., Ulvestad, E., Gjertsen, B.T., Kalland, K.-H., and Bruserud, Ø. (2005). CD34 expression in native human acute myelogenous leukemia blasts: Differences in CD34 membrane molecule expression are associated with different gene expression profiles. Cytom. Part B Clin. Cytom. *64B*, 18–27.

Pan, D., Kobayashi, A., Jiang, P., Ferrari de Andrade, L., Tay, R.E., Luoma, A.M., Tsoucas, D., Qiu, X., Lim, K., Rao, P., et al. (2018). A major chromatin regulator determines resistance of tumor cells to T cell-mediated killing. Science *359*, 770–775.

Pan, H., Gray, R., Braybrooke, J., Davies, C., Taylor, C., McGale, P., Peto, R., Pritchard,

K.I., Bergh, J., Dowsett, M., et al. (2017). 20-Year Risks of Breast-Cancer Recurrence after Stopping Endocrine Therapy at 5 Years. N. Engl. J. Med. *377*, 1836–1846.

Pardoll, D.M. (2012). The blockade of immune checkpoints in cancer immunotherapy. Nat. Rev. Cancer *12*, 252–264.

Park, G.T., Lim, S.E., Jang, S.I., and Morasso, M.I. (2002). Suprabasin, a novel epidermal differentiation marker and potential cornified envelope precursor. J. Biol. Chem. *277*, 45195–45202.

Patel, S.J., Sanjana, N.E., Kishton, R.J., Eidizadeh, A., Vodnala, S.K., Cam, M., Gartner, J.J., Jia, L., Steinberg, S.M., Yamamoto, T.N., et al. (2017). Identification of essential genes for cancer immunotherapy. Nature *548*, 537–542.

Perri, F., Longo, F., Della Vittoria Scarpati, G., Pisconti, S., Longo, V., Addeo, R., Carducci, F., Buonerba, C., Fulciniti, F., and Solla, R. (2018). Metastatic HPV-related oropharyngeal carcinoma cured with chemoradiotherapy: importance of pretherapy biomolecular assessment. Clin. Case Reports *6*, 56–62.

Pillay, J., Tak, T., Kamp, V.M., and Koenderman, L. (2013). Immune suppression by neutrophils and granulocytic myeloid-derived suppressor cells: similarities and differences. Cell. Mol. Life Sci. *70*, 3813–3827.

Pisco, A.O., Brock, A., Zhou, J., Moor, A., Mojtahedi, M., Jackson, D., and Huang, S. (2013). Non-Darwinian dynamics in therapy-induced cancer drug resistance. Nat. Commun. *4*, 1–11.

Pliml, J., and Šorm, F. (1964). Synthesis of a 2-deoxy-D-ribofuranosyl-5-azacytosine. Collect. Czechoslov. Chem. Commun. *29*, 2576–2578.

Della Porta, M.G., Gallì, A., Bacigalupo, A., Zibellini, S., Bernardi, M., Rizzo, E., Allione, B., van Lint, M.T., Pioltelli, P., Marenco, P., et al. (2016). Clinical Effects of Driver Somatic Mutations on the Outcomes of Patients With Myelodysplastic Syndromes Treated With Allogeneic Hematopoietic Stem-Cell Transplantation. J. Clin. Oncol. *34*, 3627–3637.

del Pozo Martin, Y., Park, D., Ramachandran, A., Ombrato, L., Calvo, F., Chakravarty, P., Spencer-Dene, B., Derzsi, S., Hill, C.S., Sahai, E., et al. (2015). Mesenchymal Cancer Cell-Stroma Crosstalk Promotes Niche Activation, Epithelial Reversion, and Metastatic

Colonization. Cell Rep. *13*, 2456–2469.

Pribyl, M. (2020). Supresorové buňky odvozené z myeloidní řady. Myelodysplastic Syndr. News *8*, 16–25.

Pribyl, M., Hubackova, S., Moudra, A., Vancurova, M., Polackova, H., Stopka, T., Jonasova, A., Bokorova, R., Fuchs, O., Stritesky, J., et al. (2020). Aberrantly elevated suprabasin in the bone marrow as a candidate biomarker of advanced disease state in myelodysplastic syndromes. Mol. Oncol. *14*, 2403–2419.

Prince, M.E., Sivanandan, R., Kaczorowski, A., Wolf, G.T., Kaplan, M.J., Dalerba, P., Weissman, I.L., Clarke, M.F., and Ailles, L.E. (2007). Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Proc. Natl. Acad. Sci. *104*, 973–978.

Pusztai, L., Wagner, P., Ibrahim, N., Rivera, E., Theriault, R., Booser, D., Symmans, F.W., Wong, F., Blumenschein, G., Fleming, D.R., et al. (2005). Phase II study of tariquidar, a selective P-glycoprotein inhibitor, in patients with chemotherapy-resistant, advanced breast carcinoma. Cancer *104*, 682–691.

Radisky, D.C., Levy, D.D., Littlepage, L.E., Liu, H., Nelson, C.M., Fata, J.E., Leake, D., Godden, E.L., Albertson, D.G., Angela Nieto, M., et al. (2005). Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. Nature *436*, 123–127.

Reigstad, C.S., Hultgren, S.J., and Gordon, J.I. (2007). Functional genomic studies of uropathogenic Escherichia coli and host urothelial cells when intracellular bacterial communities are assembled. J. Biol. Chem. *282*, 21259–21267.

Restifo, N.P., Marincola, F.M., Kawakami, Y., Taubenberger, J., Yannelli, J.R., and Rosenberg, S.A. (1996). Loss of Functional Beta2-Microglobulin in Metastatic Melanomas From Five Patients Receiving Immunotherapy. JNCI J. Natl. Cancer Inst. *88*, 100–108.

Revathidevi, S., and Munirajan, A.K. (2019). Akt in cancer: Mediator and more. Semin. Cancer Biol. *59*, 80–91.

Rhee, I., Bachman, K.E., Park, B.H., Jair, K.-W., Yen, R.-W.C., Schuebel, K.E., Cui, H., Feinberg, A.P., Lengauer, C., Kinzler, K.W., et al. (2002). DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. Nature *416*, 552–556.

Ribas, A., Lawrence, D., Atkinson, V., Agarwal, S., Miller, W.H., Carlino, M.S., Fisher, R., Long, G. V., Hodi, F.S., Tsoi, J., et al. (2019). Combined BRAF and MEK inhibition with PD-1 blockade immunotherapy in BRAF-mutant melanoma. Nat. Med. *25*, 936–940.

Roberts, A.W., Ma, S., Kipps, T.J., Coutre, S.E., Davids, M.S., Eichhorst, B., Hallek, M., Byrd, J.C., Humphrey, K., Zhou, L., et al. (2019). Efficacy of venetoclax in relapsed chronic lymphocytic leukemia is influenced by disease and response variables. Blood *134*, 111–122.

Rodriguez, P.C., Quiceno, D.G., Zabaleta, J., Ortiz, B., Zea, A.H., Piazuelo, M.B., Delgado, A., Correa, P., Brayer, J., Sotomayor, E.M., et al. (2004). Arginase I Production in the Tumor Microenvironment by Mature Myeloid Cells Inhibits T-Cell Receptor Expression and Antigen-Specific T-Cell Responses. Cancer Res. *64*, 5839–5849.

Romano, A., Parrinello, N.L.L., Vetro, C., Forte, S., Chiarenza, A., Figuera, A., Motta, G., Palumbo, G.A.A., Ippolito, M., Consoli, U., et al. (2015). Circulating myeloid-derived suppressor cells correlate with clinical outcome in Hodgkin Lymphoma patients treated up-front with a risk-adapted strategy. Br. J. Haematol. *168*, 689–700.

Roser, M., and Ritchie, H. (2015). Causes of Death. Https://Ourworldindata.Org/Causes-of-Death Our World Data.

Roth, G.A., Abate, D., Abate, K.H., Abay, S.M., Abbafati, C., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdela, J., Abdelalim, A., et al. (2018). Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet *392*, 1736–1788.

Ruff, P., Vorobiof, D.A., Jordaan, J.P., Demetriou, G.S., Moodley, S.D., Nosworthy, A.L., Werner, I.D., Raats, J., and Burgess, L.J. (2009). A randomized, placebo-controlled, double-blind phase 2 study of docetaxel compared to docetaxel plus zosuquidar (LY335979) in women with metastatic or locally recurrent breast cancer who have received one prior chemotherapy regimen. Cancer Chemother. Pharmacol. *64*, 763–768.

Russo, M., Crisafulli, G., Sogari, A., Reilly, N.M., Arena, S., Lamba, S., Bartolini, A., Amodio, V., Magrì, A., Novara, L., et al. (2019). Adaptive mutability of colorectal cancers in response to targeted therapies. Science (80-.). *366*, 1473–1480.

Sabeh, F., Shimizu-Hirota, R., and Weiss, S.J. (2009). Protease-dependent versus-independent cancer cell invasion programs: Three-dimensional amoeboid movement revisited. J. Cell Biol. *185*, 11–19.

Sade-Feldman, M., Kanterman, J., Klieger, Y., Ish-Shalom, E., Olga, M., Saragovi, A., Shtainberg, H., Lotem, M., and Baniyash, M. (2016). Clinical Significance of Circulating CD33+CD11b+HLA-DR- Myeloid Cells in Patients with Stage IV Melanoma Treated with Ipilimumab. Clin. Cancer Res. *22*, 5661–5672.

Sakai, T., Toguchida, J., Ohtani, N., Yandell, D.W., Rapaport, J.M., and Dryja, T.P. (1991). Allele-specific hypermethylation of the retinoblastoma tumor-suppressor gene. Am. J. Hum. Genet. *48*, 880–888.

Sallman, D.A., and List, A. (2019). The central role of inflammatory signaling in the pathogenesis of myelodysplastic syndromes. Blood *133*, 1039–1048.

Santini, V. (2019). How I treat MDS after hypomethylating agent failure. Blood *133*, 521–529.

Santini, V., Kantarjian, H.M., and Issa, J.-P. (2001). Changes in DNA Methylation in Neoplasia: Pathophysiology and Therapeutic Implications. Ann. Intern. Med. *134*, 573.

Schoenfeld, A.J., and Hellmann, M.D. (2020). Acquired Resistance to Immune Checkpoint Inhibitors. Cancer Cell *37*, 443–455.

Schwitalla, S., Fingerle, A.A., Cammareri, P., Nebelsiek, T., Göktuna, S.I., Ziegler, P.K., Canli, O., Heijmans, J., Huels, D.J., Moreaux, G., et al. (2013). Intestinal Tumorigenesis Initiated by Dedifferentiation and Acquisition of Stem-Cell-like Properties. Cell *152*, 25–38.

Seitz, H.K., and Stickel, F. (2007). Molecular mechanisms of alcohol-mediated carcinogenesis. Nat. Rev. Cancer *7*, 599–612.

Shah, N.N., and Fry, T.J. (2019). Mechanisms of resistance to CAR T cell therapy. Nat. Rev. Clin. Oncol. *16*, 372–385.

Shao, C., Tan, M., Bishop, J.A., Liu, J., Bai, W., Gaykalova, D.A., Ogawa, T., Vikani, A.R., Agrawal, Y., Li, R.J., et al. (2012). Suprabasin Is Hypomethylated and Associated with Metastasis in Salivary Adenoid Cystic Carcinoma. PLoS One *7*, 1–7.

Shen, H., and Laird, P.W. (2013). Interplay between the Cancer Genome and Epigenome. Cell *153*, 38–55.

Shervington, A., and Lu, C. (2008). Expression of Multidrug Resistance Genes in Normal and Cancer Stem Cells. Cancer Invest. *26*, 535–542.

Shiozawa, Y., Havens, A.M., Pienta, K.J., and Taichman, R.S. (2008). The bone marrow niche: habitat to hematopoietic and mesenchymal stem cells, and unwitting host to molecular parasites. Leukemia *22*, 941–950.

Short, S., Fielder, E., Miwa, S., and von Zglinicki, T. (2019). Senolytics and senostatics as adjuvant tumour therapy. EBioMedicine *41*, 683–692.

Silverman, L.R., Holland, J.F., Weinberg, R.S., Alter, B.P., Davis, R.B., Ellison, R.R., Demakos, E.P., Cornell, C.J., Carey, R.W., and Schiffer, C. (1993). Effects of treatment with 5-azacytidine on the in vivo and in vitro hematopoiesis in patients with myelodysplastic syndromes. Leukemia *7 Suppl 1*, 21–29.

Silverman, L.R., Demakos, E.P., Peterson, B.L., Kornblith, A.B., Holland, J.C., Odchimar-Reissig, R., Stone, R.M., Nelson, D., Powell, B.L., DeCastro, C.M., et al. (2002). Randomized Controlled Trial of Azacitidine in Patients With the Myelodysplastic Syndrome: A Study of the Cancer and Leukemia Group B. J. Clin. Oncol. *20*, 2429–2440.

Sinha, P., Clements, V.K., and Ostrand-Rosenberg, S. (2005). Reduction of Myeloid-Derived Suppressor Cells and Induction of M1 Macrophages Facilitate the Rejection of Established Metastatic Disease. J. Immunol. *174*, 636–645.

Sinha, P., Clements, V.K., Bunt, S.K., Albelda, S.M., and Ostrand-Rosenberg, S. (2007). Cross-Talk between Myeloid-Derived Suppressor Cells and Macrophages Subverts Tumor Immunity toward a Type 2 Response. J. Immunol. *179*, 977–983.

Sippel, T.R., White, J., Nag, K., Tsvankin, V., Klaassen, M., Kleinschmidt-DeMasters, B.K., and Waziri, A. (2011). Neutrophil Degranulation and Immunosuppression in Patients with GBM: Restoration of Cellular Immune Function by Targeting Arginase I. Clin. Cancer Res. *17*, 6992–7002.

Šorm, F., Pískala, A., Čihák, A., and Veselý, J. (1964). 5-Azacytidine, a new, highly effective cancerostatic. Experientia *20*, 202–203.

Sosa, M.S., Bragado, P., and Aguirre-Ghiso, J.A. (2014). Mechanisms of disseminated cancer cell dormancy: An awakening field. Nat. Rev. Cancer *14*, 611–622.

Spranger, S., Bao, R., and Gajewski, T.F. (2015). Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. Nature *523*, 231–235.

Srivastava, M.K., Sinha, P., Clements, V.K., Rodriguez, P., and Ostrand-Rosenberg, S. (2010). Myeloid-Derived Suppressor Cells Inhibit T-Cell Activation by Depleting Cystine and Cysteine. Cancer Res. *70*, 68–77.

Strauss, D.C., and Thomas, J.M. (2010). Transmission of donor melanoma by organ transplantation. Lancet Oncol. *11*, 790–796.

Stupp, R., Mason, W.P., van den Bent, M.J., Weller, M., Fisher, B., Taphoorn, M.J.B., Belanger, K., Brandes, A.A., Marosi, C., Bogdahn, U., et al. (2005). Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma. N. Engl. J. Med. *352*, 987–996.

Sucker, A., Zhao, F., Real, B., Heeke, C., Bielefeld, N., Massen, S., Horn, S., Moll, I., Maltaner, R., Horn, P.A., et al. (2014). Genetic Evolution of T-cell Resistance in the Course of Melanoma Progression. Clin. Cancer Res. *20*, 6593–6604.

Sumimoto, H., Imabayashi, F., Iwata, T., and Kawakami, Y. (2006). The BRAF–MAPK signaling pathway is essential for cancer-immune evasion in human melanoma cells. J. Exp. Med. *203*, 1651–1656.

Suri, A., Saini, S., Sinha, A., Agarwal, S., Verma, A., Parashar, D., Singh, S., Gupta, N., and Jagadish, N. (2012). Cancer testis antigens: A new paradigm for cancer therapy. Oncoimmunology *1*, 1194–1196.

Swann, J.B., and Smyth, M.J. (2007). Immune surveillance of tumors. J. Clin. Invest. *117*, 1137–1146.

Szakács, G., Annereau, J.-P., Lababidi, S., Shankavaram, U., Arciello, A., Bussey, K.J., Reinhold, W., Guo, Y., Kruh, G.D., Reimers, M., et al. (2004). Predicting drug sensitivity and resistance. Cancer Cell *6*, 129–137.

Szmulewitz, R., Taylor, J., and Rinker-Schaffer, C. (2015). Metastatic Colonization. Encycl. Cancer *529*, 2797–2799.

Tabasso, A.F.S., Jones, D.J.L., Jones, G.D.D., and Macip, S. (2019). Radiotherapy-Induced Senescence and its Effects on Responses to Treatment. Clin. Oncol. *31*, 283–289.

Takahashi, K., Asano, N., Imatani, A., Kondo, Y., Saito, M.M., Takeuchi, A., Jin, X., Saito, M.M., Hatta, W., Asanuma, K., et al. (2020). Sox2 induces tumorigenesis and angiogenesis of early-stage esophageal squamous cell carcinoma through secretion of Suprabasin. Carcinogenesis *26*, 1–15.

Tausch, E., Close, W., Dolnik, A., Bloehdorn, J., Chyla, B., Bullinger, L., Döhner, H., Mertens, D., and Stilgenbauer, S. (2019). Venetoclax resistance and acquired BCL2 mutations in chronic lymphocytic leukemia. Haematologica *104*, e434–e437.

Teeuwssen, and Fodde (2019). Wnt Signaling in Ovarian Cancer Stemness, EMT, and Therapy Resistance. J. Clin. Med. *8*, 1658.

The Royal College of Radiologists (2019). Radiotherapy dose fractionation, third edition. Clin. Oncol. *12*, 17.

Thiery, J.P., Acloque, H., Huang, R.Y.J., and Nieto, M.A. (2009). Epithelial-Mesenchymal Transitions in Development and Disease. Cell *139*, 871–890.

Tsai, H.-C., Li, H., Van Neste, L., Cai, Y., Robert, C., Rassool, F. V., Shin, J.J., Harbom, K.M., Beaty, R., Pappou, E., et al. (2012). Transient Low Doses of DNA-Demethylating Agents Exert Durable Antitumor Effects on Hematological and Epithelial Tumor Cells. Cancer Cell *21*, 430–446.

Tu, C.-C., and Hsu, P.-K. (2018). The frontline of esophageal cancer treatment: questions to be asked and answered. Ann. Transl. Med. *6*, 83–83.

Venturelli, S., Berger, A., Weiland, T., Essmann, F., Waibel, M., Nuebling, T., Hacker, S., Schenk, M., Schulze-Osthoff, K., Salih, H.R., et al. (2013). Differential Induction of Apoptosis and Senescence by the DNA Methyltransferase Inhibitors 5-Azacytidine and 5-Aza-2'-Deoxycytidine in Solid Tumor Cells. Mol. Cancer Ther. *12*, 2226–2236.

Vermeulen, L., De Sousa E Melo, F., van der Heijden, M., Cameron, K., de Jong, J.H., Borovski, T., Tuynman, J.B., Todaro, M., Merz, C., Rodermond, H., et al. (2010). Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nat. Cell Biol. *12*, 468–476.

Vitale, I., Manic, G., Coussens, L.M., Kroemer, G., and Galluzzi, L. (2019). Macrophages and Metabolism in the Tumor Microenvironment. Cell Metab. *30*, 36–50.

Vlashi, E., and Pajonk, F. (2015). Cancer stem cells, cancer cell plasticity and radiation therapy. Semin. Cancer Biol. *31*, 28–35.

Wang, Z., and Cao, Y.J. (2020). Adoptive Cell Therapy Targeting Neoantigens: A Frontier for Cancer Research. Front. Immunol. *11*, 176.

Wang, L., Amoozgar, Z., Huang, J., Saleh, M.H., Xing, D., Orsulic, S., and Goldberg, M.S. (2015a). Decitabine Enhances Lymphocyte Migration and Function and Synergizes with CTLA-4 Blockade in a Murine Ovarian Cancer Model. Cancer Immunol. Res. *3*, 1030–1041.

Wang, S., Song, R., Wang, Z., Jing, Z., Wang, S., and Ma, J. (2018). S100A8/A9 in Inflammation. Front. Immunol. *9*, 1298.

Wang, Y., He, L., Du, Y., Zhu, P., Huang, G., Luo, J., Yan, X., Ye, B., Li, C., Xia, P., et al. (2015b). The Long Noncoding RNA lncTCF7 Promotes Self-Renewal of Human Liver Cancer Stem Cells through Activation of Wnt Signaling. Cell Stem Cell *16*, 413–425.

Wang, Z., Zhang, L., Wang, H., Xiong, S., Li, Y., Tao, Q., Xiao, W., Qin, H., Wang, Y., and Zhai, Z. (2015c). Tumor-induced CD14+HLA-DR−/low myeloid-derived suppressor cells correlate with tumor progression and outcome of therapy in multiple myeloma patients. Cancer Immunol. Immunother. *64*, 389–399.

Weber, J., Gibney, G., Kudchadkar, R., Yu, B., Cheng, P., Martinez, A.J.J., Kroeger, J., Richards, A., McCormick, L., Moberg, V., et al. (2016). Phase I/II Study of Metastatic Melanoma Patients Treated with Nivolumab Who Had Progressed after Ipilimumab. Cancer Immunol. Res. *4*, 345–353.

Whiteside, T.L. (2008). The tumor microenvironment and its role in promoting tumor growth. Oncogene *27*, 5904–5912.

Wijermans, P., Krulder, J., Huijgens, P., and Neve, P. (1997). Continuous infusion of low-dose 5-Aza-2′-deoxycytidine in elderly patients with high-risk myelodysplastic syndrome. Leukemia *11*, 1–5.

Wijermans, P., Lübbert, M., Verhoef, G., Bosly, A., Ravoet, C., Andre, M., and Ferrant, A.

(2000). Low-Dose 5-Aza-2′-Deoxycytidine, a DNA Hypomethylating Agent, for the Treatment of High-Risk Myelodysplastic Syndrome: A Multicenter Phase II Study in Elderly Patients. J. Clin. Oncol. *18*, 956–956.

Williams, E.D., Gao, D., Redfern, A., and Thompson, E.W. (2019). Controversies around epithelial–mesenchymal plasticity in cancer metastasis. Nat. Rev. Cancer *19*, 716–732.

Van Willigen, W.W., Bloemendal, M., Gerritsen, W.R., Schreibelt, G., De Vries, I.J.M., and Bol, K.F. (2018). Dendritic cell cancer therapy: Vaccinating the right patient at the right time. Front. Immunol. *9*, 1–13.

Winter, S., Shoaie, S., Kordasti, S., and Platzbecker, U. (2020). Integrating the "Immunome" in the Stratification of Myelodysplastic Syndromes and Future Clinical Trial Design. J. Clin. Oncol. *38*, 1723–1735.

Wrangle, J., Wang, W., Koch, A., Easwaran, H., Mohammad, H.P., Pan, X., Vendetti, F., VanCriekinge, W., DeMeyer, T., Du, Z., et al. (2013). Alterations of immune response of non-small cell lung cancer with Azacytidine. Oncotarget *4*, 2067–2079.

Wu, C., Wu, X., Zhang, X., Chai, Y., Guo, Q., Li, L., Yue, L., Bai, J., Wang, Z., and Zhang, L. (2015). Prognostic significance of peripheral monocytic myeloid-derived suppressor cells and monocytes in patients newly diagnosed with diffuse large b-cell lymphoma. Int. J. Clin. Exp. Med. *8*, 15173–15181.

Wylie, B., Chee, J., Forbes, C.A., Booth, M., Stone, S.R., Buzzai, A., Abad, A., Foley, B., Cruickshank, M.N., and Waithman, J. (2019). Acquired resistance during adoptive cell therapy by transcriptional silencing of immunogenic antigens. Oncoimmunology *8*, 1609874.

Xu, J., Lamouille, S., and Derynck, R. (2009). TGF-β-induced epithelial to mesenchymal transition. Cell Res. *19*, 156–172.

Yaguchi, T., Goto, Y., Kido, K., Mochimaru, H., Sakurai, T., Tsukamoto, N., Kudo-Saito, C., Fujita, T., Sumimoto, H., and Kawakami, Y. (2012). Immune Suppression and Resistance Mediated by Constitutive Activation of Wnt/β-Catenin Signaling in Human Melanoma Cells. J. Immunol. *189*, 2110–2117.

Yang, H., Bueso-Ramos, C., DiNardo, C., Estecio, M.R., Davanlou, M., Geng, Q.-R.,

Fang, Z., Nguyen, M., Pierce, S., Wei, Y., et al. (2014). Expression of PD-L1, PD-L2, PD-1 and CTLA4 in myelodysplastic syndromes is enhanced by treatment with hypomethylating agents. Leukemia *28*, 1280–1288.

Yang, L., DeBusk, L.M., Fukuda, K., Fingleton, B., Green-Jarvis, B., Shyr, Y., Matrisian, L.M., Carbone, D.P., and Lin, P.C. (2004). Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor-bearing host directly promotes tumor angiogenesis. Cancer Cell *6*, 409–421.

Yang, L., Qian, Y., Eksioglu, E., Epling-Burnette, P.K., and Wei, S. (2015). The inflammatory microenvironment in MDS. Cell. Mol. Life Sci. *72*, 1959–1966.

Yasuda, Y., Ozasa, H., Kim, Y.H., Yamazoe, M., Ajimizu, H., Yamamoto Funazo, T., Nomizo, T., Tsuji, T., Yoshida, H., Sakamori, Y., et al. (2020). MCL1 inhibition is effective against a subset of small-cell lung cancer with high MCL1 and low BCL-XL expression. Cell Death Dis. *11*, 177.

Yuan, W., Yuan, Y., Zhang, T., and Wu, S. (2015). Role of Bmi-1 in Regulation of Ionizing Irradiation-Induced Epithelial-Mesenchymal Transition and Migration of Breast Cancer Cells. PLoS One *10*, e0118799.

Yumoto, K., Eber, M.R., Wang, J., Cackowski, F.C., Decker, A.M., Lee, E., Nobre, A.R., Aguirre-Ghiso, J.A., Jung, Y., and Taichman, R.S. (2016). Axl is required for TGF-β2-induced dormancy of prostate cancer cells in the bone marrow. Sci. Rep. *6*, 36520.

Zahalka, A.H., and Frenette, P.S. (2020). Nerves in cancer. Nat. Rev. Cancer *20*, 143–157.

Zaretsky, J.M., Garcia-Diaz, A., Shin, D.S., Escuin-Ordinas, H., Hugo, W., Hu-Lieskovan, S., Torrejon, D.Y., Abril-Rodriguez, G., Sandoval, S., Barthly, L., et al. (2016). Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma. N. Engl. J. Med. *375*, 819–829.

Zhu, J., Wu, G., Li, Q., Gong, H., Song, J., Cao, L., Wu, S., Song, L., and Jiang, L. (2016). Overexpression of Suprabasin is Associated with Proliferation and Tumorigenicity of Esophageal Squamous Cell Carcinoma. Sci. Rep. *6*, 21549.

Zimmer, L., Livingstone, E., Hassel, J.C., Fluck, M., Eigentler, T., Loquai, C., Haferkamp, S., Gutzmer, R., Meier, F., Mohr, P., et al. (2020). Adjuvant nivolumab plus ipilimumab or

nivolumab monotherapy versus placebo in patients with resected stage IV melanoma with no evidence of disease (IMMUNED): a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet *395*, 1558–1568.

12. The publications