

# Cancer immunology makes it to clinic

how cancer will be treated  
in the coming years







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VIII Forum

26 September 2015, Lugano

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# Presentation

## **Silvia Misiti**

Head of IBSA Foundation for Scientific Research

## **Giuseppe Zizzo**

Secretary of IBSA Foundation for Scientific Research

The Forum “Cancer immunology makes it to clinic: how cancer will be treated in the coming years” was organized by IBSA Foundation for scientific research together with Dr. Andrea Alimonti, Head of the Molecular Oncology Lab (Southern Switzerland Cancer Institute, Bellinzona), in collaboration with the Bellinzona research institutions and USI.

The Forum – attended by around 200 participants (students, researchers, medical specialists, etc.) – brought together prominent scientist from all over the world who have given a significant contribution to this important scientific breakthrough. The program, ranged from clinics to basic immunology, favoured wide discussions.

Immunotherapy has opened a new era in cancer treatment, so much so that the journal “Science” has put it at the top of its list of the ten most important scientific breakthroughs of recent years. Fighting cancer through cells that are part of our body is the revolutionary concept underlying this game-changing treatment approach. While chemotherapy and radiotherapy target the tumour, immunotherapy awakens and enhances the patient’s immune system inducing it to attack tumour cells from within. Since one of the main feature of the immune system is its heterogeneity, sharing promising results obtained with different approaches by different laboratories through over the world could extremely help to have a complete overview of the entire picture.





# Introduction

## Andrea Alimonti

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For more than 50 years, scientists have been trying to harness the patient immune system against cancer. But decades of failures have revealed that tumours have the ability to evade, tamp down and overwhelm the normal immune response.

However, recent immune therapies have been proved to be extremely effective in treating several different types of cancer. These therapies try to educate the immune system to recognize and attack tumour cells and have been named “checkpoints” inhibitors. In 2011 the FDA has approved the first checkpoint inhibitor, ipilimumab for the cure of malignant melanoma. Additional trials suggest that drugs that block a different checkpoint, called PD-1, are even more effective and have fewer side effects than ipilimumab.

In recent studies, checkpoint blockades produced improvements in between 20% and 65% of patients, depending on the drug, dosage and type of cancer. Early research suggests that ipilimumab may be even more effective when combined with other drugs and in the future several combination will be tested in cancer patient with either targeted and conventional therapies (e.g. chemotherapy, radiotherapy).

Given the excitement behind these results, the 2013 have been designated by the journal Science the year of cancer immunotherapy. However several questions remained unanswered. For instance we still do not know how the genetic background of the patients tumors can shape the tumour microenvironment and the tumour immune response. Another issue is to understand how to stratify patients suitable for the different types immunotherapies and more importantly to understand how patients develop resistance to these treatments during time.

Objective of this meeting was to bring together scientists and clinicians at the forefront of cancer biology and therapy to get a deeper insight on the current revolutions of cancer immunotherapy.



## SESSION 1



# The Tumor Immune Landscaping Project & The Co-clinical trial Platform

**Pier Paolo Pandolfi**

Cancer Center, Cancer Research Institute, Department of Medicine and Pathology, Division of Genetics, Harvard Medical School & Beth Israel Deaconess Medical Center, Boston, USA

The Co-Clinical Trial Project takes advantage of a new platform for cancer clinical trial optimization [1, 2]. The Project also rests on a “Mouse Hospital” infrastructure [3], which is equipped as a human hospital to perform experimental clinical trials in mouse models of disease, exactly as they would be run in the human hospital.

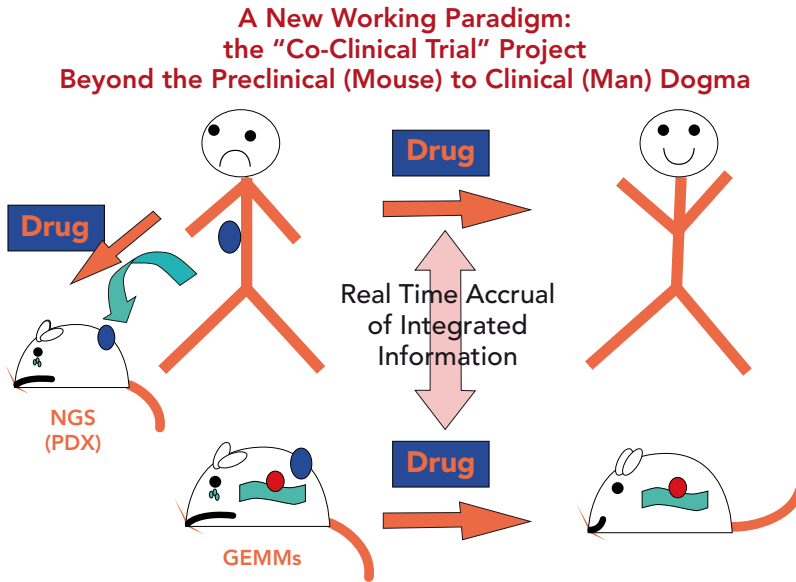
In the “Co-Clinical” Approach for Cancer Therapy optimization, mouse models of cancer, which are representative of the diversity of human cancer, are treated with the same drug, and following the very same clinical protocol offered to human patients enrolled in experimental clinical trials in the human hospital. This allows for “mice-to-human” stratification and cross-validation of response and resistance to specific treatment modalities (• **Figure 1**).

In the “Mouse Hospital”, drugs can be tested on “immune-deficient” mice (nude, NGS or other models) that are transplanted with human tumors derived from biopsy (patient-derived xenographs, PDX). Importantly, a “Co-Clinical” protocol can also be run, in parallel, enrolling “immune-competent” genetically engineered mouse models of cancer (GEMMs) bearing genetic mutations associated to human cancer. Hence the “Co-Clinical Platform” is a three-pronged approach with Human Patients, PDXs, and GEMMs simultaneously enrolled in clinical trials for analysis.

The implementation of co-clinical trials, beyond the pre-clinical and clinical dogma, allows for real-time integrated genetic stratifications of response. This offers the possibility to rapidly stratify the responses to a drug, and to identify novel biomarkers, as well as mechanism of resistance. The Co-Clinical Trial is currently ongoing in Boston at the BIDMC Cancer Center of Harvard Medical School and in other laboratories.

The new platform can also be used to determine the “immune landscape” within the tumor microenvironment of cancers (tumor immune landscape or TME for brief), to understand whether differential composition of TME impacts on tumorigenesis and

- **Figure 1.** The co-clinical trial project for the development of precision medicine and personalized care



The co-clinical paradigm aims to develop mouse trials in parallel with human clinical trials to enable rapid, real-time transfer of information from mouse experiments to human trials and allows optimization and improved outcomes of clinical efforts. For patients that present in the clinic, co-clinical trials can facilitate evaluation of treatment in a number of ways. Patient tumours can be engrafted in NSG immunodeficient mice to generate PDX models of the tumour. Alternatively, appropriate GEMMs can be identified that act as a surrogate for the patient, based on the genetics of the patient's tumour. While patients themselves are enrolled in clinical trials, PDX and GEMMs models can be enrolled in co-clinical trials carried out in parallel. Sensitivity of tumours to the same drugs, novel drugs or combinations thereof can be rapidly evaluated in these co-clinical trials, and real time integration of data between clinical and co-clinical trials can inform patient treatment protocols.

GEMM, genetically engineered mouse model; NSG, NOD scid  $\gamma$ ; PDX, patient-derived xenograft.

thus to identify novel therapeutic entries. GEMMs of various tumor types can be used to address these points. In order to evaluate immune infiltration, immunophenotypic analyses can be performed from the tumors.

Preliminary data indicate that different genetic backgrounds trigger differential recruitment of immune cells by the tumors. These immune cells in turn produce different inflammatory and pro-tumoral molecules that impact tumorigenesis and dictate different outcomes.

Importantly, these results are fully translatable to the human clinic. Indeed, patients carrying the same tumor suppressor deletions/mutation engineered in mice were found to express higher level of the same chemokine that has been observed to modify the TME in GEMMS (unpublished data).

In conclusion, the Co-Clinical Trial proves informative at multiple levels: it reveals that immune landscape in prostate cancer differentially affects tumorigenesis on the basis of its genetic features with important therapeutic implications. Hence different cancer genetic make-ups differentially drive the development of prostate cancer, in turn generating different immune milieus, and thus requiring different therapeutic strategies.

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# Immunology and lymphomas

**Franco Cavalli**

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Non Hodgkin Lymphomas (NHL) represent as a group of neoplasias the fifth most common tumor type in North America and western Europe, whereby the incidence has doubled in the last thirty years. In the past one of the main problems in dealing with NHL was the fact that there was no classification which was universally accepted, since all classifications were based only on morphology and it was impossible to have all pathologists agreeing in order to have just one sole way of classifying these neoplastic disorders. Recently, however, a new classification (Revised European American Lymphoma classification, REAL) was developed based not only on morphology, but also on genetic, immunophenotypic, epidemiological and clinical data. After a first discussion at the International Conference on malignant Lymphomas in Lugano (1996) an international validation study was organized, which led to the universal acceptance of the REAL classification, which in the meantime has become the WHO classification [1].

Coming to the treatment, we can verify in all databases, that the outcome in the vast majority of the B-cell NHL has improved after the year 2000, in coincidence largely with the introduction in the various treatment approaches of the anti-CD20 monoclonal antibody.

While at least 50% of the most common subtype of NHL (Diffuse B Cell Lymphomas, DLBCL) can be cured, the indolent variants (follicular lymphomas (FL), marginal zone lymphomas and small lymphocytic lymphomas) remain still incurable, although the life expectancy of the patients has greatly improved: in follicular lymphomas, e.g., the median life expectancy of newly diagnosed cases of follicular lymphoma is today in the order of 18 years. However, the disease remains incurable even with the most aggressive approach, which means the use of high dose chemotherapy and autologous transplantation in first-line treatment [2].

It has been demonstrated, that the micro-environment plays an important role in determining the outcome of patients with follicular lymphoma: therefore a further improvement in the immunotherapeutic approach to this subtype of lymphoma might possibly be one of the components of a therapeutic approach, which could envisage cure as the ultimate goal.

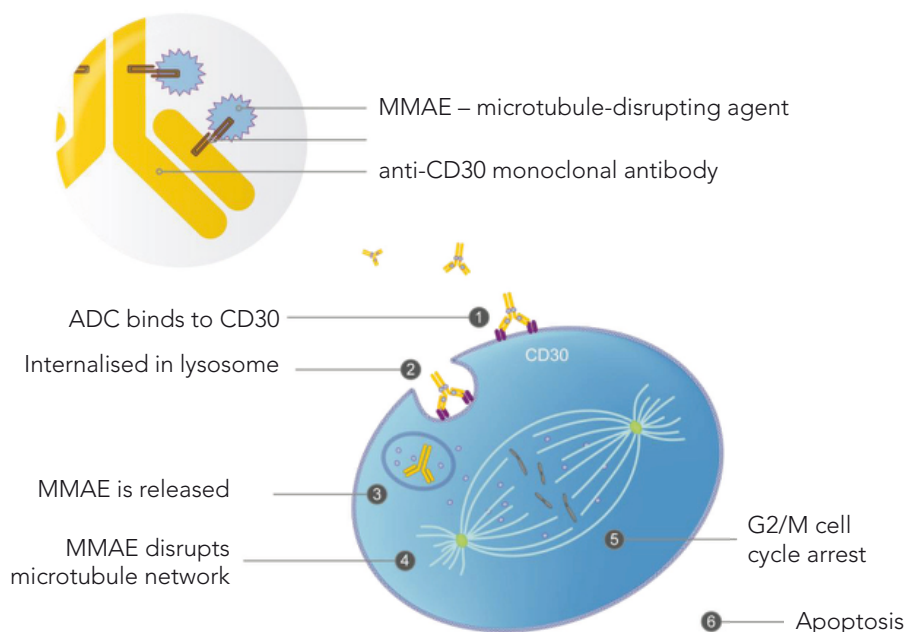
Marginal zone lymphoma (MZL) are characterised by different cell of origin, different genetic abnormalities and clinical behaviours. All of them are however always associated with a chronic antigen stimulation, either in the context of an autoimmune disease or because of a chronic infection. The paradigmatic example is gastric MALT lymphoma, which is elicited by a chronic infection by *H. Pylori*. Other infectious agents can elicit MALT lymphomas in other localisations. Besides an approach with antibiotics to eliminate infectious agents, if MZL become resistant to this approach or relapse as a systemic disease, the best results are achieved with a combination of anti-CD20 monoclonal antibodies and the cytotoxic agent chlorambucil [3].

Between malignant lymphomas and immunology there is of course a “natural connection”, since lymphocytes represent the backbone of our immunologic defence mechanism. For a long period of time, the only immunotherapeutic approach which was available was represented by the allogeneic stem cell transplantation prompting a graft-vs-host reaction. Today immunotherapy means for lymphomas the use of monoclonal antibodies and recently the engagement of T-cells. The Swiss experience has shown that at least half of the patients with FL can be initially treated with anti-CD20 (mabthera) alone, whereby the progression free survival can be prolonged by the use of a maintenance treatment after the induction therapy. The fact that half of these patients are still in remission at about 8 years after having been treated with mabthera alone [4], prompted many investigators to start developing chemotherapy free approaches, the so-called R<sup>2</sup>. Recently the Swiss group has demonstrated that the combination of mabthera with lenalidomide can elicit statistically significant superior results as compared with mabthera alone (unpublished data).

Positive results were achieved also with the so-called radio-immunotherapeutic agents: however, this remains a complex and expensive technology [5]. The next step was the introduction of conjugated antibodies in the treatment plan of many lymphoma subtypes. For instance, an anti-CD30 has been conjugated with a microtubule-disrupting agent and this complex has achieved very high response rates both in Hodgkin Lymphoma (HL) as well as in some T-cell lymphomas, which express a high level of CD30. In this situation the anti-CD30 antibodies drive the anti-tubule agent inside the cells, leading to cell apoptosis (• **Figure 1**). In patients with HL a very high response rate has been achieved even in those relapsing after stem cell transplantation.

A part of monoclonal antibodies, it has been observed that IFN treatment prolongs survival of FL patients. Therefore, a strategy based on the combined use of

• **Figure 1.** Anti-CD30 conjugated with anti-tubulin agent mechanism of action



Anti-CD30 monoclonal antibodies conjugated with MMAE are internalized in lysosome, where MMAE is released and act on microtubule network.

IFN and chemotherapy has been proposed [6]. However, it is important to take in account that high doses of IFN lead to cytotoxic events instead of immunomodulating ones, thus it cannot be considered as real immunotherapy.

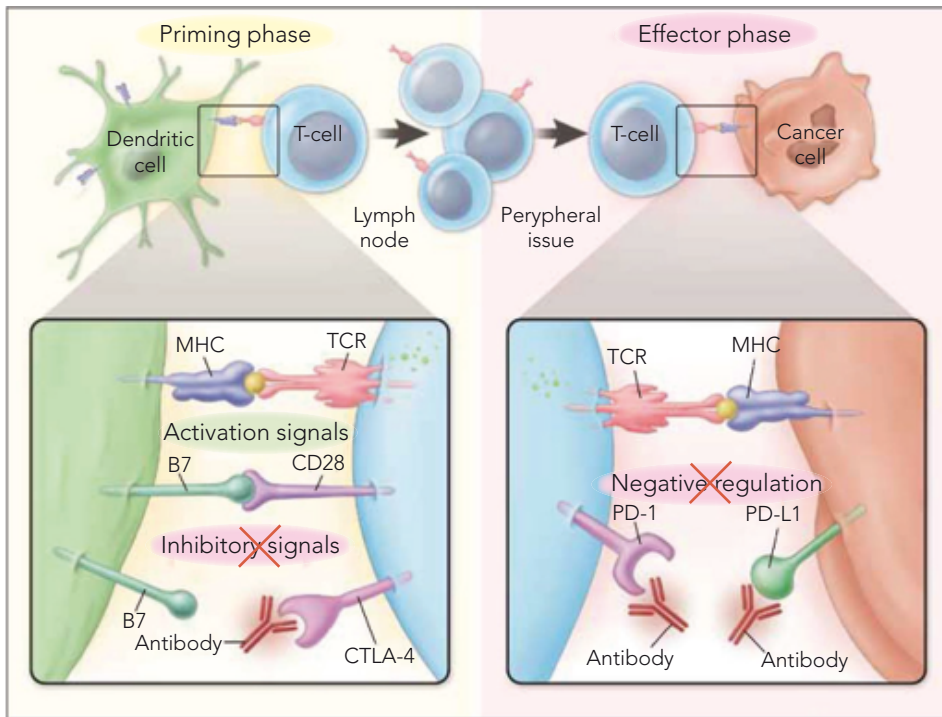
Another possible approach is the use of vaccine. It has been observed that FL patients that develop anti-idiotypic antibodies have a longer response after chemotherapy [7].

Finally, checkpoint inhibitors are one of the most promising approaches. Anti-CTLA-4, anti-PD-1 and anti-PD-L1 block T cell activation and incorporation of ligands leading to tolerance [8] (• **Figure 2**).

The response of lymphoma patients to anti-PD1 was not satisfactory as only a small percentage of the cohort improves the outcome. Therefore, a combined therapy based on anti-PD1 and anti-CD20 was developed for FL patients. The treatment leads to ameliorated outcome for 50% of the patients [9].

Another approach is the use of chimeric antigen receptors (CAR)-T cells. This therapy is based on T cell bearing anti-CD19 ligands, that target malignant B cells

• **Figure 2.** Checkpoint inhibitor mechanism of action



Anti-CTLA or PD-L1 antibodies by preventing the binding with their receptors on dendritic cells or T cells respectively avoid the generation of inhibitory signals or negative regulation, favoring tumor cell recognition by immune cells.

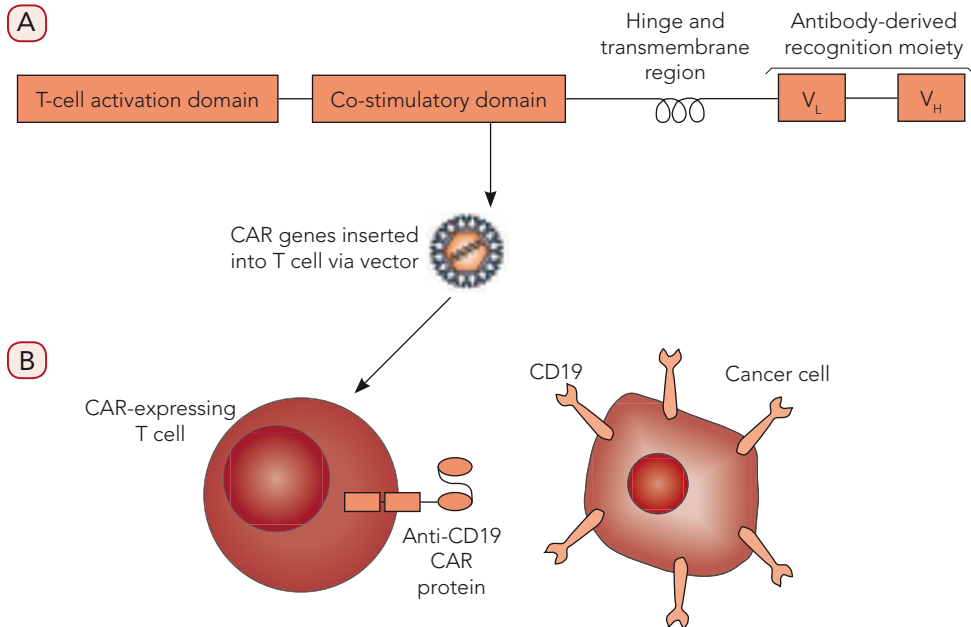
Source: modified from Ribas, 2012 [8].

[10] (• **Figure 3**). Although this therapy gave good results in pediatric patients, it was not efficient for adults [11].

Finally, bi-specific antibodies have been generated. These are antibodies connecting also T cells. They resulted active in the treatment of acute lymphoblastic leukemia but they had no important application in malignant lymphomas [12].

To conclude, anti-PD-L1 therapies were efficient in the treatment of CLL in mice models, suggesting the possible use of this agent in clinics. It represents an example of tumor interacting with the microenvironment [13].

• **Figure 3.** Chimeric antigen receptor T cell mechanism of action



T cells are loaded with anti-CD19 CAR protein that target malignant B cells.

Source: modified from Kochenderfer, Rosenberg, 2013 [10].

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# Surprising roles of lymphatic endothelium in cancer progression and inflammation

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Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology, ETH Zurich, Switzerland

Although cells of the immune system play an important role in the control of cancer development and progression, one also needs to investigate the role of resident cells, such as lymphatic and endothelial cells, that have even become therapeutic targets. Detection of sentinel lymph nodes is a method that is used every day in hospitals, and detection of lymph node metastases increases the risk for cancer progression and further metastasis. Therefore, it is important to understand the mechanisms by which cancer cells metastasize to lymph nodes and beyond.

Lymphatic vessels drain the fluids from tissues through capillaries. Cancer cells can induce lymphatic vessel growth and thereby promote lymph node metastasis. Accordingly, tumor-induced lymphangiogenesis has been shown to predict overall survival of patients affected by melanoma and other types of cancer, including breast, colorectal and lung cancer. Lymphatic vessels might also provide an immunoprotected cancer stem cell niche. Indeed, “initiating” cancer cells can survive for several years in lymphatic vessels. Tumors can also induce lymph node lymphangiogenesis that even occurs before tumor metastasization and that favors the generation of a pro-metastatic niche, preparing for the arrival of metastatic cells. As a consequence, tumor and lymph node lymphangiogenesis actively promote cancer metastasis.

In order to better understand the mechanisms by which lymphatic cells promote tumor progression and impair patient survival, murine models of metastasis have been used. In a model of metastasis driven by 4T1 breast cancer cells, an early expansion of tumor draining lymph nodes was observed, before cancer cells were detected. Importantly, recent studies indicate that lymphatic vessels can induce CD4 and CD8 T cell tolerance, with possible implications for the inhibition of anti-tumor immune responses.

In order to better understand whether lymphatic endothelial cells in expanded lymph nodes might be different from lymphatic endothelial cells of normal ones, a



transcriptome profiling of FACS-sorted tumor associated lymphatic vessels was performed. A number of molecules were identified to be upregulated on tumor-associated lymphatic endothelium. These could be used as markers for *in vivo* imaging, but some of these also appear to be involved in the suppression of immune responses.

Recently, the dogma stating that tumor metastases are derived from the primary tumor mass has been challenged. Indeed, it has been found that primary tumors generate metastases and that these metastases can generate other metastases. They might even metastasize to lymph nodes and then from lymph nodes to other organs [1]. Thus, the metastatic process is much more complicated than previously thought.

In order to understand whether lymphatic endothelium plays a role also in this metastasis-to-metastasis generation, a characterization of lung and skin metastases derived from human melanomas has been performed and there is evidence for lymphatic expansion in some of these metastases, with detection of lymphatic invasion. These findings indicate that pharmacological inhibitors of tumor lymphangiogenesis might represent a novel therapeutic strategy in the clinics. Two drugs are already tested in early stage clinical studies, namely monoclonal antibodies against VEGF-C or its receptor VEGFR-3, but the field is completely open for additional inhibitors.

To assess the efficacy of therapeutic drugs against lymphangiogenesis, it is important to develop non-invasive new tools to visualize lymphatic vessels and to evaluate whether the drugs are indeed hitting their targets. Through *in vivo* imaging, it is possible to visualize and quantify the draining rate, the pumping rate and the contractility of lymphatic vessels, and to compare it in treated versus untreated tumor settings. Sophisticated fluorescent reporter mouse models represent new tools to study lymphatic vessels *in vivo*.

Thanks to these techniques, it is now possible to also study the role of lymphatic vessels in chronic inflammatory diseases and thus to investigate whether they are anti- or pro-inflammatory in this context. It has been observed that lymphatic vessel drainage is impaired in chronic skin inflammation.

Accordingly, the blockade of lymphatic vessel function with an anti-VEGFR3 antibody prolonged edema formation and inflammation in a model of psoriasis. Therefore, it was hypothesized that therapeutic agents that re-establish lymphatic vessel activity in inflammatory diseases might have beneficial effects. Indeed, the overexpression of VEGF-C in the skin of K14-VEGF-C transgenic mice inhibited inflammation and the same occurred when the protein (VEGF-C) was injected into the skin. Similar results were obtained in a collagen-induced model of rheumatoid arthritis and also in a model of inflammatory bowel disease when VEGF-C was applied.

Together, these results clearly suggest that the delivery of VEGF contributes to resolving inflammation. Thus, molecules have been developed that promote the lymphatic function in inflamed tissues after systemic application. These molecules potentially inhibit chronic skin inflammation.

In conclusion, lymphatic vessels promote the formation of a pre-metastatic niche and also enhance metastasis, thereby representing a new therapeutic target. Lymphatic



vessel function is impaired in chronic inflammation and the delivery of lymphatic vessel inducers promotes the drainage and reduces inflammation.

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# Regulation of the senescence-associated secretory phenotype (SASP)

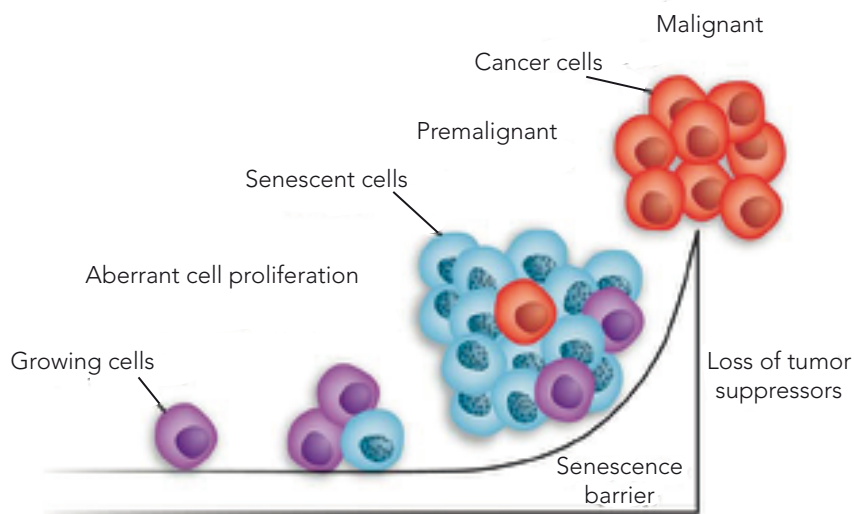
Jesús Gil

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Senescence is a phenomenon that affects primary cells going through limited replicative potential. Senescence can be caused by several issues, included telomere dysfunction, oxidative stress, DNA damage, cytotoxic drugs and cancer. Indeed, oncogene activation can lead to senescence and oncogene-induced senescence is a barrier for cancer: aberrant cell proliferation can develop in growing cells, leading to the generation of premalignant senescent cells and then to malignant cancer cells [1] (• **Figure 1**). Senescence represents a stable arrest of cell growth, characterized by a typical phenotype: enlarged cells  $\beta$ -gal positive with senescence-associated heterochromatin foci and senescence-associated secretory phenotype (SASP). Moreover, p53 and p16 are key players in inducing senescence.

An *in vitro* system of oncogene-induced senescence has been developed. It consists in an inducible system of human fibroblasts expressing chimeric ER:RAS protein: upon tamoxifen treatment, cells became p21, p53 and p16 positive and they activate DNA damage response, ROS production and SASP. This system has been used to perform a SILAC analysis of SASP that allows the identification of the molecules included in the SASP [2]. SASP is a combination of multiple pro-inflammatory factors: cytokines, chemokines and growth factors that influence the micro-environment. Focusing on cancer, SASP display either pro-tumoral or anti-tumoral features. Indeed, on one hand SASP promotes epithelial-to-mesenchymal transition, tumor invasion, proliferation and angiogenesis, and on the other hand it is able to activate both innate and adaptive immunity and to reinforce senescence and paracrine senescence. Through a mouse model of liver senescence, it has been demonstrated that SASP recalls CD4 T lymphocytes that, together with monocytes and macrophages, eliminate senescent cells by a mechanism called “senescence surveillance”, limiting liver cancer development [3]. Therefore, SASP represents a good therapeutically target for cancer.

• **Figure 1.** Oncogene-induced senescence is a barrier for cancer



Growing cells going through aberrant proliferation became premalignant senescent cells, that in absence of tumor suppressors develop in malignant cancer cells.

Source: Narita, Lowe, 2005 [1].

SASP can reinforce and induce senescence, as a paracrine effect on other cells. Indeed, by exposing normal cells to SASP through a co-culture with senescent cells in transwell, it has been observed that normal cells became senescent [2].

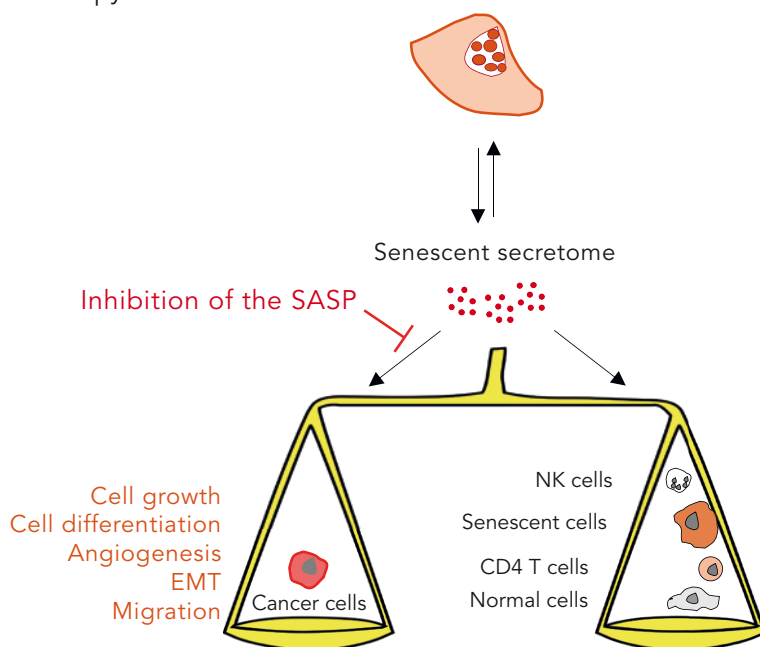
These results have been confirmed also in an *in vivo* model of cancer [2]. Since multiple compounds mediate this phenomenon, included CCL2, TGF- $\beta$  and IL-8, it is impossible to identify a “single drug” that can be used to target paracrine senescence. Nevertheless, it can be possible to act on SASP regulation. Indeed, it is known that SASP have both pro-tumoral and anti-tumoral characteristics, thus by inhibiting the pro-tumoral activity, it can be possible to improve and sustain the anti-tumoral one (• **Figure 2**).

In order to understand how SASP transcription is regulated, a screening of 32 chemical compounds that potentially modulate SASP has been performed. It emerged that SASP can be controlled by NF $\kappa$ B, inflammasome inhibitors and mTOR inhibitors [2].

Further experiments confirmed an involvement of IL-1 signalling in the regulation of SASP production and senescence. Since mTOR is known to integrate upstream signal and control cellular processes such as protein translation, lipid synthesis or autophagy, a secretome analysis upon mTOR blocking was performed [4].

It has been observed that different mTOR inhibitors reduced the transcription of different molecules composing senescent secretome without rescuing the cell arrest

• **Figure 2.** The relationship between senescent secretome and cancer as possible target for therapy



SASP displays both pro-tumoral and anti-tumoral activities. By interfering with pro-tumoral activities it is possible to drive the anti-tumoral ones.

associated to senescence [4]. It suggests that mTOR inhibition dissociates the growth arrest from SASP production. Among different transcriptional factors downstream mTOR, it has been observed that 4EBP plays a major role in regulating SASP [4]. Since the association of mRNAs with a 5' TOP with polysomes depends of mTOR/4EBP, it suggests that translation and not transcription is involved in this process [4]. Accordingly, it has been demonstrated that MK2 translation is regulated by mTOR/4EBP and its inhibition affects the SASP [4]. Downstream, the mRNA binding protein regulating stability of mRNAs with AU-rich elements BRF1/ZFP36L1 is phosphorylated by MK2 during senescence [4]. Accordingly, it has been demonstrated that BRF1/ZFP36L1 directly controls the SASP [4].

To summarize, mTOR inhibitors by blocking mTOR prevent 4EBP-mediated activation of MK2 and then of BRF1/ZFP36L1, avoiding SASP production. It remains to define whether this pathway has a role in tumor promoting or tumor suppressing features of SASP.

It has been observed that mTOR inhibitors restore the cell proliferative capacity *in vitro* and reduce p21 and p16 expression in pre-neoplastic hepatocytes *in vivo*. There-

fore, mTOR is involved in the positive regulation of paracrine senescence and thus in the anti-tumoral effects of SASP [4]. Interestingly, it has been observed that mTOR inhibitors affect E-cadherin expression on cells treated with senescent cells-conditioned medium and favor tumor growth in *in vivo* model of cancer, thus suggesting that mTOR plays a role also in controlling the pro-tumoral features of SASP [4].

In conclusion, mTOR inhibitors can dissociate the growth arrest from SASP production on senescent cells and thus can be considered for suppress the “bad” effects of SASP. Nevertheless, it is important to underline that mTOR inhibitors are immuno-suppressive and they prevent cell growth through mechanism unrelated to senescence. Therefore, it is necessary to keep looking for different strategies to target SASP.

In order to address this point, a screening of SASP regulation using HCA and siRNA libraries was performed (unpublished data). It emerged that 49 genes that were knock down by siRNA are involved in SASP inhibition without rescuing the senescent growing arrest (unpublished data). Among them, at least 9 can be targeted by commercial drugs, thus they represent good candidates for possible therapies (unpublished data).

All together, these results highlight the importance of SASP as mediator of effects of senescent cells and suggest how its components and regulation can be targeted for therapeutic benefit.

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# A non-cell-autonomous mechanism controlling senescence evasion and chemoresistance in tumors

Andrea Alimonti

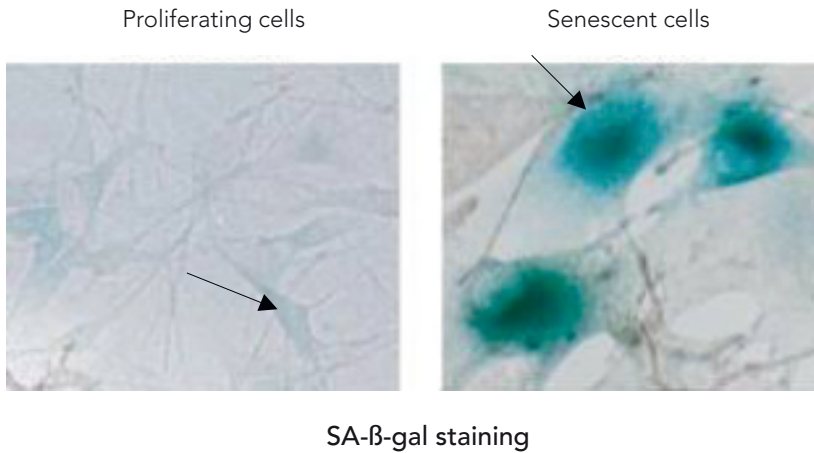
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Senescence is an irreversible cell growth arrest that opposes tumour proliferation [1]. At least two kind of senescence can be identified: replicative senescence, caused by telomere attrition, and premature senescence, that occurs upon oncogene activation, DNA damage or suboptimal growth conditions. Senescence cells are characterised by large nuclei and a strong positivity to SA- $\beta$ -galactosidase staining (● **Figure 1**). It has been demonstrated that PTEN-loss-induced cellular senescence (PICS) represents a senescence response that is distinct from oncogene-induced senescence and that can be targeted for cancer therapy [1]. There are many compounds that enhance senescence in cancer currently tested in clinical trials. Compound that enhance senescence in cancer do not only promote an irreversible growth arrest but can also recruit and activate immune cells, favouring cancer clearance.

It has been observed that PTEN KO tumors are characterized either by a proliferative and a senescence cellular compartment. Interestingly we have found that Gr-1<sup>+</sup> infiltrates can oppose PTEN loss-induced cellular senescence [2]. This granulocyte-mediated effect was due to alteration in IL-1 $\alpha$  signaling [2]. In particular, MDSCs by producing IL-1Ra block the IL-1 $\alpha$  receptor signaling, thus avoiding cellular senescence.

The same mechanism occurs in adenocarcinoma areas in lung tumors from K-ras<sup>+/G12V</sup> mice where Gr-1<sup>+</sup> infiltrates, opposes senescence and enhance proliferation. Interestingly, it has been observed that PTEN KO tumors expressed high levels of CXCL2, suggesting that CXCL2-CXCR2 axis could be involved in granulocyte recruitment. Indeed, anti-CXCR2 treatment reduced tumor size, that was further reduced when anti-CXCR2 was combined with the pro-senescence agent Docetaxel. Accordingly, anti-CXCR2 treatment induced senescence markers at level comparable to docetaxel [2]. These results are translatable to humans as patients

• **Figure 1.** SA- $\beta$ -gal staining identifies senescent cells



Proliferating cells are SA- $\beta$ -gal negative compared to senescent cells that can be recognized by SA- $\beta$ -gal positivity.

Source: Alimonti lab.

with increased percentage of CD33+ IL-1Ra+ myeloid infiltrates have a reduced response to docetaxel, suggesting a possible role of IL-1Ra as biomarker.

It is a matter of fact that patients bearing tumors expressing MDSC-associated genes are more susceptible to tumour relapses. Indeed, the presence of a myeloid signature and of circulating myeloid cells correlate with poor prognosis in prostate cancer patients. Therefore, CXCR2 inhibitors could be good candidate for clinical trials in humans.

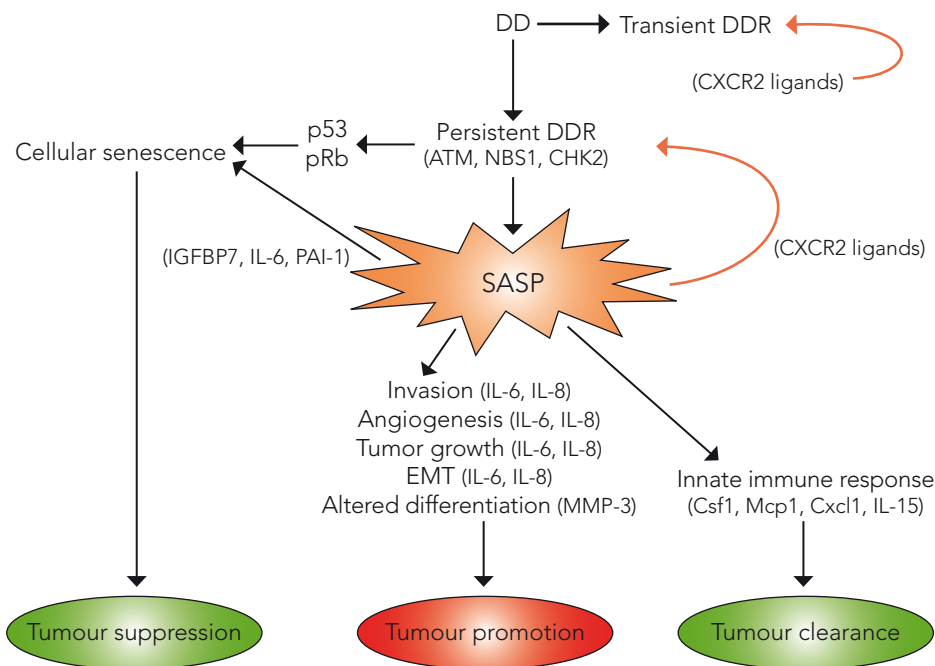
Macrophages are immune cell belonging to the myeloid compartment. Then, the role of macrophages in PTEN/p53 KO animals has been recently investigated from our lab. It has been observed that a consistent percentage of macrophages that infiltrate the tumours display a M2 phenotype (unpublished data). Interestingly, compound that block the M1-M2 polarization promote growth arrest and senescence in PTEN/p53 KO tumors (unpublished data).

In order to understand the mechanism underlying this phenomenon, an *in vitro* model has been developed. PTEN KO MEFs were treated with M1 or M2-conditioned medium. Interestingly, M1-conditioned medium reduced proliferation of MEFs and induced  $\beta$ -gal positivity, suggesting that M1 favor senescence in PTEN KO cells. Accordingly, PTEN KO MEFs treated with M1-conditioned media re-activated p21 (unpublished data). Other experiments are ongoing to study the relationship between senescence, macrophages and tumors.

Another approach to enhance the efficacy of senescence therapy in cancer is to re-program the senescence associated secretory phenotype (SASP). It is known that the SASP plays a dual role in tumorigenesis: on one hand it contributes to tumor suppression by favoring senescence and by activating immune cells and on the other hand through cytokines that modulate invasion, angiogenesis and EMT, it promotes tumor growth (• **Figure 2**). Whether the SASP can be reprogrammed is unknown.

To address this question, we have performed an *in vivo* profile of the SASP of PICS. We have observed that the SASP of PTEN null senescent tumours is sustained by the activation of the Jak2/Stat3 pathway [3]. Stat3 is known to promote an immune suppressive microenvironment through the recruitment of myeloid cells and inhibition of CD3 positive cells. Accordingly, Stat3 deletion in PTEN null tumours prevented the immunosuppressive phenotype of the PICS SASP [3]. The impairment of the immunosuppressive phenotype correlated with re-activation of immune cells, in particular

• **Figure 2.** Dual role of the senescence associated secretory phenotype (SASP) in tumorigenesis



SASP can act as tumor suppressive element by favoring cellular senescence and by activating immune system. Conversely, SASP is involved also in tumor promotion, by favoring invasion, angiogenesis, tumor growth and EMT.

Source: Alimonti lab.



CD4 and CD8 T cells, NK cells and B cells, improving their capability to release anti-tumoral factors [3]. The combination of docetaxel with JAK2 inhibitors resulted in strong anti-tumor response mediated by CD3 and granzyme B positive cells [3]. Therefore, Stat3 represents a regulator of SASP that can be targeted to reprogram the SASP from an immunosuppressive to an immune-promoting phenotype.

All together these results highlight a strong relationship between senescence and immune system in cancer that can be targeted for cancer therapy.

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# Immunity and prostate cancer

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For several years, the value of immunotherapy remained unproven, despite much work to find circulating tumor biomarkers, by sequencing plasma or by interrogating circulating tumour cells in blood. Through mRNA profiling of all blood coming from cancer patients, it is possible to observe that the most represented mRNA species are driven from myeloid cells. Metastatic prostate cancer patients have a whole blood mRNA signature that indicates a substantially increased myeloid cell population in blood and a decreased lymphocyte population. Such a signature associates with a higher neutrophil to lymphocyte ratio, which associates with a poorer prognosis from advanced prostate cancer and many other tumour types. These clinical data mirror what has been described in transgenic murine models where increased myeloid suppressor cell populations have been described, impacting tumour growth. Critically, data are accumulating that established therapeutic work best when the patient has low levels of myeloid cells, suggesting that the immune system is an important mediator of cancer therapeutic resistance. This supports the targeting of the immune system to reverse treatment resistance. It has been postulated that the tumour cell is the conductor of the immune response, driving an immune response that enhances tumour growth. Strategies targeting cancer cells and the immune system are now needed to improve patient outcome.

Metastatic prostate cancer is the most common cancer in man; advanced prostate cancer patients are now living longer with improved treatment options. This is a tumor mainly driven by hormones and the therapeutic value to androgen deprivation is well described. Several new treatments have recently been approved that improve outcome from this disease including sipuleucel T, docetaxel, cabazitaxel, abiraterone, radium-223 and enzalutamide. Sipeuleucel T is not widely used, at least in part due to the costs of administering this agent. These drugs have all been developed for all

metastatic prostate cancer patients; molecular stratification is not routinely currently utilized for treatment administration. Many different molecular subtypes of this disease have however been reported. These include tumours with a mismatch repair defect (1-5%), for whom PD1/PD-L1 therapies need to be evaluated. Importantly, we have now shown that 30% of sporadic metastatic prostate cancers have DNA repair defects and that patients bearing these mutations respond to PARP inhibitors.

In conclusion, the treatment of prostate cancer is changing rapidly. Identified DNA repair defects in up to 30% of these tumours make new therapeutic strategies including treatment with PARP inhibitors and immunotherapies a real possibility. Furthermore, blood based immune biomarkers can inform not only on patient prognosis but also on response to both hormonal and cytotoxic therapy. Such biomarkers will be useful in the development of novel therapeutic strategies for the treatment of this disease.

## SESSION 2



# Breaking barriers to effective immunotherapy against solid tumors

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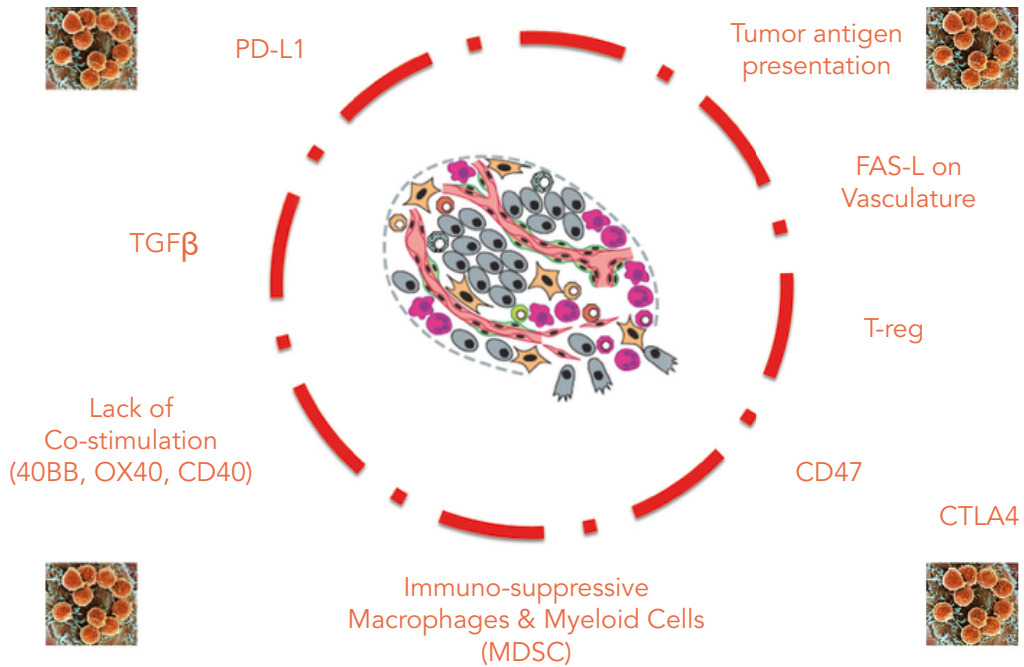
The most important aim of immunotherapy is to manipulate immune system to attack cancer. One of the “next generation” hallmarks of cancer is “avoiding immune cell destruction” [1] and checkpoint inhibitors anti-CTLA4 and anti-PD-1/PD-L1 work exactly in this direction.

It has been reported that the anti-CTLA4 ipilimumab as well as nivolumab (anti-PD-1) improves survival of melanoma patients [2, 3], demonstrating the effective efficiency of these treatments. Notably, many patients (~half) do not respond to anti-CTLA4 or anti-PD-1 administration, suggesting the presence of intrinsic or adaptive resistance to checkpoint inhibitor therapy. This evident resistance is suspected to be due to the fact that tumors can develop multiple and diverse barriers to avoid infiltration and killing by immune cells.

For instance, many solid tumors are protected by barriers that block the entry of activated cytotoxic T cells. One line of evidence comes from the RIP-Tag5 mouse model of pancreatic neuroendocrine cancer. In this model the SV40 large T antigen (Tag) is expressed under the control of an insulin gene promoter, and as a result these animals develop hyperplastic/dysplastic islets that eventually become angiogenic, progressing to solid tumors and in turn invasive carcinomas [4].

Even when the immune system is engineered such that 80% of T cells are reactive against this tumor antigen, the tumor evolves. Accordingly, T cells of transgenic mice expressing a tumor specific T cell receptor (RIP1-Tag5 x Tag-TCR2) are attracted to the tumors but are unable infiltrate, kill, and impair tumor growth [4]. At present, a number of possible barriers erected by solid tumors are implicated (• **Figure 1**), including: induction of checkpoint ligands that turn off cytotoxic T cells; lack of co-stimulatory ligands that maintain T cell activity; recruitment of regulatory (inhibitory) T cells and myeloid derived suppressive cells (MDSC); repression of formation of high

• **Figure 1.** Barriers to effective immune killing of tumors



Tumors develop barriers to avoid immune cell recognition and elimination. By breaking these barriers it may be possible to empower the immune system to kill cancers.

Source: D.H., unpublished.

endothelial venules on tumor vasculature to limit extravasation; induction of FasL on endothelial cells to kill extravasating T cells; lack of tumor antigens or impaired antigen presentation; and production of TGF- $\beta$  and other immunosuppressive factors. It remains to understand which combinations of barriers are operative in particular tumors, not how to effectively break them down.

To this aim, a mouse model of HPV16-driven cancer with high incidence of female reproductive tract cancer has been used. HPV-driven cancer is one of the best candidates for immunotherapies. Indeed, it is driven by E6 and E7 oncogenes that encode viral non-self antigens that are immunogenic. E6 binds and inactivates the p53 tumor suppressor, whereas E7 gene binds and sequesters the Rb tumor suppressor. Since invasive cervical cancer has a poor prognosis, and the prophylactic capsid vaccine is not efficient against pre-existing cancer, the E6/E7 oncoproteins could represent a good target for immunotherapies. Importantly, this model generates squamous carci-

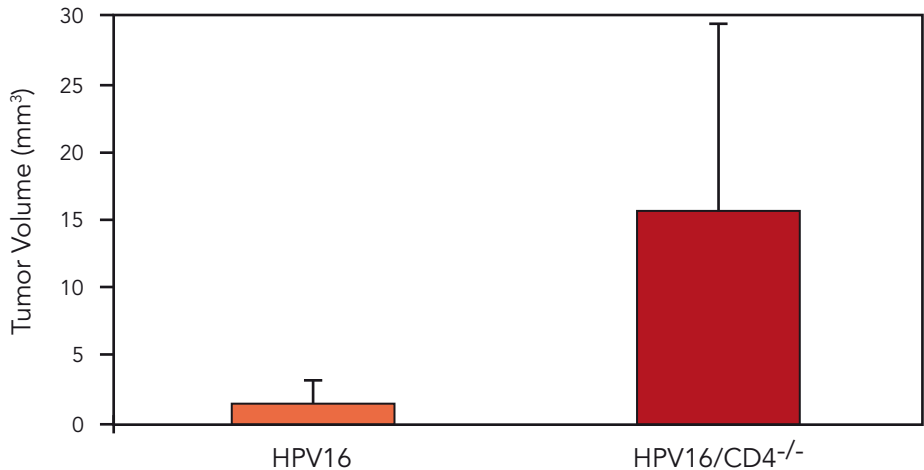
nogenesis in skin and cervix comparable to human tumors, allowing a possible direct translation to clinics. Interestingly, all HPV16 mice in the FVB/n background have skin dysplasia between 3 and 6 months of age and 50% of them develop squamous carcinoma of the skin between 6-12 months. In opposite, C57/B6 genotype is not permissive to squamous carcinogenesis. Since the E6/E7 oncoproteins of HPV16 are not presented by MHC class I of FVB/n, the first generation model represents an opportunity to assess CD4 compartment in the absence of reactive CD8.

In order to study the contribution of CD4 T cells to squamous carcinogenesis, the phenotype of K14-HPV16/CD4 KO animals was evaluated. In the absence of CD4 T cells tumor volume is increased, suggesting that the immune system is able to recognize cervical tumors, impairing but not preventing its development (● **Figure 2**) [5].

To enhance this anti-tumor effect, animals were treated with a vaccine based on E7 (HspE7), that primes E7-specific CD4 T cell and CTL response without the need of an exogenous adjuvant. It has been observed that the tumor incidence in HspE7-receiving mice is reduced and CD4 T cell are required for the efficacy of the vaccine. Indeed, in the absence of CD4 T cells (HPV16/CD4 KO mice) the vaccine has no effect [5].

Although this evidence clearly suggests that CD4 T cells are able to impair cervical cancer development and the HspE7 vaccine acts on CD4 T cells improving their capability to control tumor growth, the mechanisms underlying are unknown.

● **Figure 2.** CD4 T cell contribution to cancer formation



Cervical tumor size in HPV16 and HPV16/CD4 <sup>-/-</sup> animals.

Source: modified from Daniel et al., 2005 [5].



More recently, a second generation model has been developed, which is a better representation of the human cancer in regard to immunobiology. The HPV16 transgenic mice were rendered congenic for H2b MHC class I, which presents E7 peptides on class I MHC to CD8 T cells; the K14-HPV16/H2b mice therefore recognize the E7 oncoprotein and develop CTL response against it. The animals display the same tumoral phenotype observed in the first generation model. CD8 T cell recognition of E7 proteins has been verified by E7 immunogenicity assay (ELISpot) and *in vitro* cytotoxicity and in both cases CD8 T cells result activated upon E7 recognition.

Therefore, as performed in the first generation, a vaccine to improve CD8 T cell responses was generated. It represents a nanoparticles vaccine called NP-E7 able to activate E7-specific T cells. Even if the treatment with this vaccine increased the number of E7-specific CD8 T cells infiltrating the K14+ area, it does not reduced tumor burden (unpublished data). Interestingly, different levels of CD8 T cell infiltration were observed in different tumor region of vaccinated mice (G. Galliverti, S. Wullschleger, M. Swartz, and D.H., unpublished data).

In order to better understand this phenotype, the TC-1 cell line, which expresses E6/E7 onco-proteins, was injected subcutaneously into C57BL/6 mice to develop transplant tumors. Upon vaccine delivery, CD8 T cells of vaccinated mice infiltrate the TC-1 tumors, causing complete regression (G. Galliverti, S. Wullschleger, M. Swartz, and D.H., unpublished data). This difference clearly suggests that spontaneous tumors in HPV16 animals develop barriers that ectopic tumors are unable to create.

Several candidate barriers could be built by the tumor to avoid T cell recognition: tumor cells can express PD-L1 or FAS ligands, they could recruit MDSCs or Tregs or they can lack co-stimulatory agonists. Among them, the expression of PD-L1 seemed likely to be involved – albeit not exclusively – as HPV16 tumors express PD-L1 and E7-specific CD8 T cells of these mice express PD-1. Although the NP-E7 vaccine has been delivered together with anti-PD-1, the combination of these treatments does neither increase the number of CD8 T cell infiltrating the tumor nor decrease tumor burden, excluding a singular contribution of PD-1/PD-L1 to this process (G. Galliverti, S. Wullschleger, M. Swartz, and D.H., unpublished data). Interestingly, moving on to the myeloid compartment, it has been observed that a macrophage infiltrate surrounds the tumors in the cervix of HPV16 animals. Some of these macrophages express CD11c, others express CD206 and a third group co-express CD11c and CD206, suggesting that M1, M2 and mixed phenotypes are present (G. Galliverti, S. Wullschleger, M. Swartz, and D.H., unpublished data). Further experiments will be performed to assess macrophage contribution to tumor formation and immune suppression in cervical carcinomas of HPV16 mice.

In conclusion, it has been demonstrated that a therapeutic E7 vaccine improves T cell responses and it is efficient in targeting subcutaneous tumors. Nevertheless, it has been observed only a limited response by T cells in spontaneous tumors, arguing for the presence of tumor barriers. The PD-1/PD-L1 axis is evidently not the only mechanism

used by these tumor cells to avoid T cell recognition. However, the presence of myeloid infiltration, and in particular macrophages, suggests that myeloid cells can play an active role in resisting T cell infiltration and killing. Collectively these observations highlight the importance to elucidate mechanisms of immune resistance and develop mechanism-guided immunotherapy strategies to disrupt multiple tumor barriers operative in cervical carcinomas, in order to effectively unlock tumor immunity.

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# Immunotherapeutic control of tumor metastasis

Mark J. Smyth

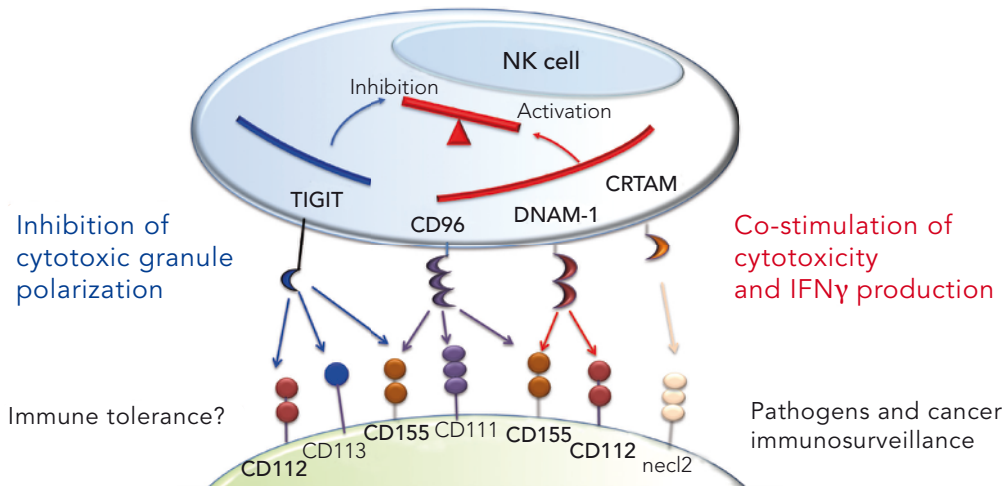
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It is accepted that immune cells are involved in metastasis control. NK cells are innate lymphocytes endowed with pro-inflammatory and anti-tumor activities that play an important role in interfering with metastasis formation. Indeed, Mcl-1-lox x Ncr1-Cre animals, which lack mature NK cells through the deletion of Mcl-1 gene, injected with B16F10 melanoma cell line develop higher number of metastases if compared to controls [1]. The activation of NK cells is regulated by a balance between activating and inhibitory receptors. Nectin receptors belong to a family of NK cell receptors that are composed by both activating and inhibitory molecules. For example, DNAM-1 and TIGIT bind the same ligands but they have opposite functions: DNAM-1 is an activating receptor that works as a co-stimulator molecule for cytotoxicity and IFN- $\gamma$  production, whereas TIGIT is involved in the inhibition of cytotoxic granule polarization and IFN- $\gamma$  production. Therefore, even if they belong to the same family, DNAM-1 participates in pathogen and cancer immunosurveillance, whereas TIGIT could favour immune tolerance [2] (● **Figure 1**).

In order to evaluate the role of nectin family receptors in metastasis formation, DNAM-1 KO, CD96 KO and TIGIT KO mice were injected with B16F10 cells and metastasis formation was assessed. DNAM-1 KO mice have increase their capability to develop metastasis, whereas CD96 KO mice have less metastasis compared to control WT mice. The number of metastases in TIGIT KO mice was comparable to that observed in WT mice [3] (● **Figure 2**).

The same results were obtained by injecting another melanoma cell line SM1WT1 [3]. To demonstrate the involvement of NK cells in this phenotype, Rag2 KO mice were transferred with CD96 KO or WT NK cells before B16F10 cell injection. CD96 KO cell transfer prevented metastasis formation compared with WT NK cell transfer [3]. These data suggest that the absence of CD96 protects from metastasis,

• **Figure 1.** Previous view of nectin family receptors



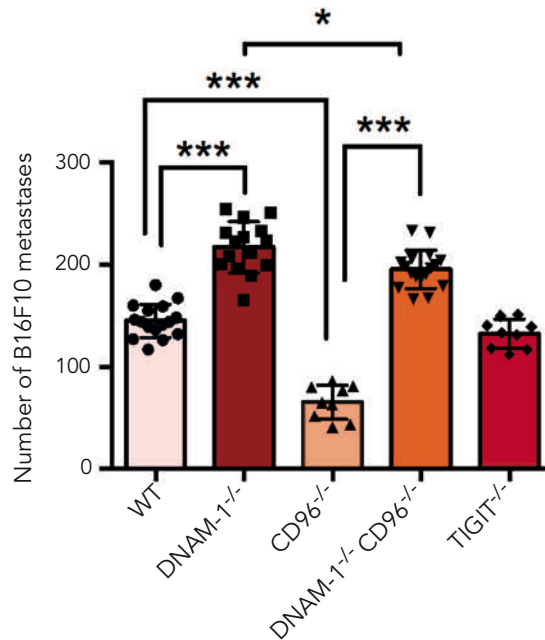
The balance between activation and inhibition in NK cells is regulated by signals coming from different receptors (activating or inhibitory receptors). The nectin family is composed by either activating or inhibitory receptors that binds the same ligands but generate opposite signals. Until recently, CD96 was believed to be a costimulator.

Source: modified from Chan *et al.*, 2014 [2].

but CD96 can also be considered as a possible target for immunotherapy. Efforts are now underway to test anti-mouse CD96 therapies in various syngeneic mouse models of metastasis and primary tumor formation. Although these are promising results, the role of anti-human CD96 has to be evaluated in human tumors. First, the capability of anti-human CD96 to rescue human lymphocytes effector functions can be achieved *in vitro*. Then, humanized anti-human CD96 can be generated. Furthermore, it is also necessary to investigate the expression of TIGIT, DNAM-1, CD96 and CD155 in mice and human tumor-microenvironment to identify the targets together with the evaluation of the role of CD96 on other lymphocyte subsets (T cells,  $\gamma\delta$  T cells and NKT cells).

It has been observed that increased levels of extracellular adenosine favour the generation of a tumor promoting microenvironment. Extracellular adenosine is a molecule derived from ATP that binds different types of adenosine receptors with different functions: the binding to A1 and A3 inhibits cAMP release, whereas the binding to A2A and A2B stimulates cAMP signalling. Adenosine receptors are expressed by immune cells, including NK cells. Adenosine has an inhibitory effect on NK cell functions, as it reduces NK cell cytotoxicity, IFN- $\gamma$  production and ma-

● **Figure 2.** Nectin 1 family contribution to metastasis formation



WT and DNAM-1, CD96, TIGIT KO mice were injected with B16F10 melanoma cell line and the number of lung metastasis were quantified.

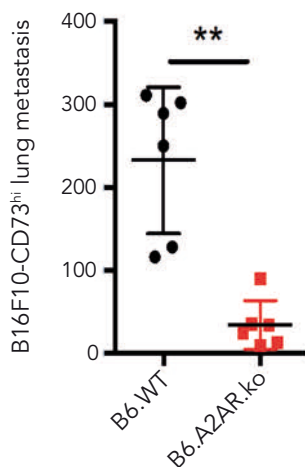
Source: modified from Chan et al., 2014 [3].

turation. It has been observed that NK cells express higher levels of A2A adenosine receptor compared to other lymphocytes and mice deficient for A2A are protected from metastasis formation (● **Figure 3**).

The same protection phenotype was observed in mice treated with A2A inhibitor [4] and the combination of A2A inhibitor with T cell checkpoint inhibitor further improves the protection [5]. Importantly, it has been demonstrated that the mechanism of action of anti-PD1 and A2ARi requires NK cells and T cells as the anti-metastatic effect is abrogated when T cells or NK cells were depleted [5].

In conclusion, these results highlight the importance of combine therapeutic strategies to improve metastatic control. In addition, they further evidence the potent immunosuppressive functions of adenosine in a tumor context, arguing for the consideration of adenosine as target for cancer immunotherapies.

● **Figure 3.** A2AR contribution to metastasis formation



WT and A2AR KO mice were injected with B16F10 melanoma cell line and the number of lung metastasis was quantified on day 14. Each symbol represents an individual mouse.

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# Heterogeneity of cancer inflammation: a double-edged sword for therapy

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Macrophages are cells belonging to the innate immune system that occupy different niches within the tumor. They can be found in the peri-vascular area, as well as in the peri-necrotic or hypoxic areas. Depending on their location and the micro-environmental stimuli, they can acquire different phenotypes, according with their main feature that is plasticity. In general, the two extremes of a continuous spectrum of activation states are represented by M1 macrophages and M2 macrophages. M1 display anti-tumoral properties, such as the capability to induce adaptive immune responses against cancer cells and to hinder angiogenesis. In opposite, M2 behave as tumor promoting cells, as they are characterized by immunosuppressive and pro-angiogenetic features [1].

Since macrophages associated to the hypoxic area of the tumor are able to block T cell response and are angiogenetic cells, they can be considered pro-tumoral macrophages [2-5]. One of the main gene responsible for the entry of macrophages in hypoxic areas is Nrp1. Nrp1 encodes for neuropilin-1, that works as co-receptor for several extracellular ligands, included VEGF (vascular endothelium growth factor) and semaphorins [3]. It has been observed that myeloid specific deletion of the Nrp1 gene (Nrp1 KO mice) prevents macrophage entry in hypoxic areas and it leads to an impairment of tumor growth and a reduction of metastasis formation [3]. Furthermore, the lack of Nrp1 inhibits angiogenesis and restores the anti-tumor response mediated by immune cells [3]. When macrophages reach hypoxic region Nrp1 is transcriptional induced and it leads to macrophage entrapment in hypoxic tumor niches. The absence of Nrp1 favors macrophage entrapment in normoxic tumor regions, leading to angiogenesis inhibition and promotion of anti-tumor immunity. On the basis of these observations, Nrp1 could be considered a possible target to “re-educate” macrophages within hypoxic areas, driving tumor-associated macrophages from a tumor-promoting to an anti-tumoral phenotype.



Macrophage metabolism could be a possible target to modulate macrophage polarization in the tumor once these macrophages have reached hypoxic regions. Indeed, different oxygen availability could affect macrophage metabolism. Furthermore, M1 and M2 macrophages display opposite metabolic signature. M1 macrophages are characterized by high glycolysis and low oxygen consumption, whereas M2 are low glycolytic cells with high oxygen consumption [2, 3, 6-8].

By performing a gene profile of macrophages treated with tumor-conditioned medium and grown *in vitro* in hypoxic conditions, it emerged that Redd1 is one of the most synergically induced gene (unpublished data).

The same result was observed in tumor-associated macrophages (TAMs) isolated by hypoxic region of established tumors (unpublished data). Redd1 is a molecule induced by cytokines or hypoxia that behaves as mTOR inhibitor. Therefore, Redd1 KO TAMs in hypoxic areas display an increased mTOR activity (unpublished data). Augmented mTOR activity leads to induced glucose uptake and glycolysis, reduced oxygen consumption and higher levels of reactive oxygen species (ROS) released by mitochondria (unpublished data).

This induction of mitochondrial ROS release is depending from induced mTOR activity but also from glycolysis-mediated NADH production [9]. According with the observation that mitochondrial ROS in immune cells can induce NF-kB activation [10], NF-kB is more activated in Redd1 KO macrophages compared to controls and it relies on mitochondrial ROS and glycolysis (● Figure 1; unpublished data).

Since NF-kB has been described to be important in switching macrophage polarization, the role of Redd1 in macrophage polarization has been investigated. Although *in vitro* T cell suppression mediated by macrophages is comparable between Redd1 KO and control mice, macrophages derived from Redd1 KO mice and co-cultured *in vitro* with endothelial cells reduce the elongation ratio of endothelial cells (unpublished data).

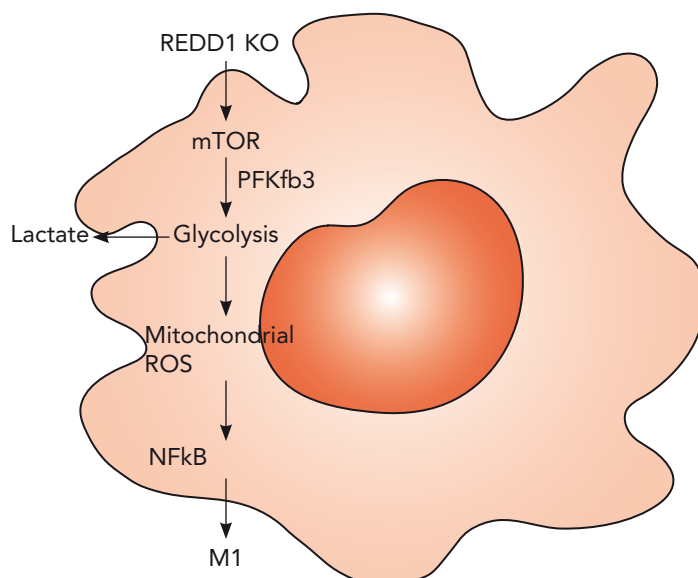
Moving from an *in vitro* to an *in vivo* approach, chimeric animals were obtained by transplanting WT or Redd1 KO bone marrow (BM) in WT recipients then injected subcutaneously with Lewis lung cell carcinoma (LLC). The metabolic features of TAMs in Redd1 KO animals were comparable to the ones observed *in vitro* that resulted in tumor blood vessel normalization and reduction of the hypoxic region compared to controls (unpublished data).

According with the idea that cancer cells have more difficulties to reach the circulation if the vasculature is normalized [2, 11, 12], Redd1 KO chimeras are characterized by a reduced number of circulating cancer cells, a reduced number of metastasis and reduced metastatic burden (unpublished data).

The same results have been obtained by the use of an orthotopic and spontaneous models of breast cancer (unpublished data). The block of glycolysis in Redd1 KO TAMs allows the restoration of the pro-angiogenic properties of endothelial cells *in vitro* and *in vivo* (unpublished data).



● **Figure 1.** Metabolic changes in REDD1 KO macrophages **drive** macrophage polarization



In conclusion, it has been demonstrated that Redd1 is overexpressed in TAMs. High levels of Redd1 strongly inhibit mTOR activation, leading to reduced glycolysis and ROS production and thus favouring an M2-like phenotype. If Redd1 is abolished, mTOR is activated, glycolysis and ROS production are increased and macrophages acquire an anti-tumor M1-like phenotype. All together these results suggested that Redd1, by modulating metabolic changes, could affect macrophage polarization and thus could be considered a potential target to re-educate TAMs.

From a clinical point of view, mTOR inhibitors are available and are used in clinical trials and they usually give good results in inhibiting cancer cell growth. Indeed, they impair cell survival and proliferation. However, it is important to pay attention to the fact that mTOR inhibitors could also impact on stromal cells. The results obtained by our group clearly show how mTOR inhibition leads to reduced glycolysis and ROS production, and therefore impaired M1 polarization favouring an M2-like pro-tumoral phenotype. Further experiments have to be done, but these results highlight an important issue for the use of mTOR inhibitors in clinics.

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# Inflammation and cancer from bench to bedside

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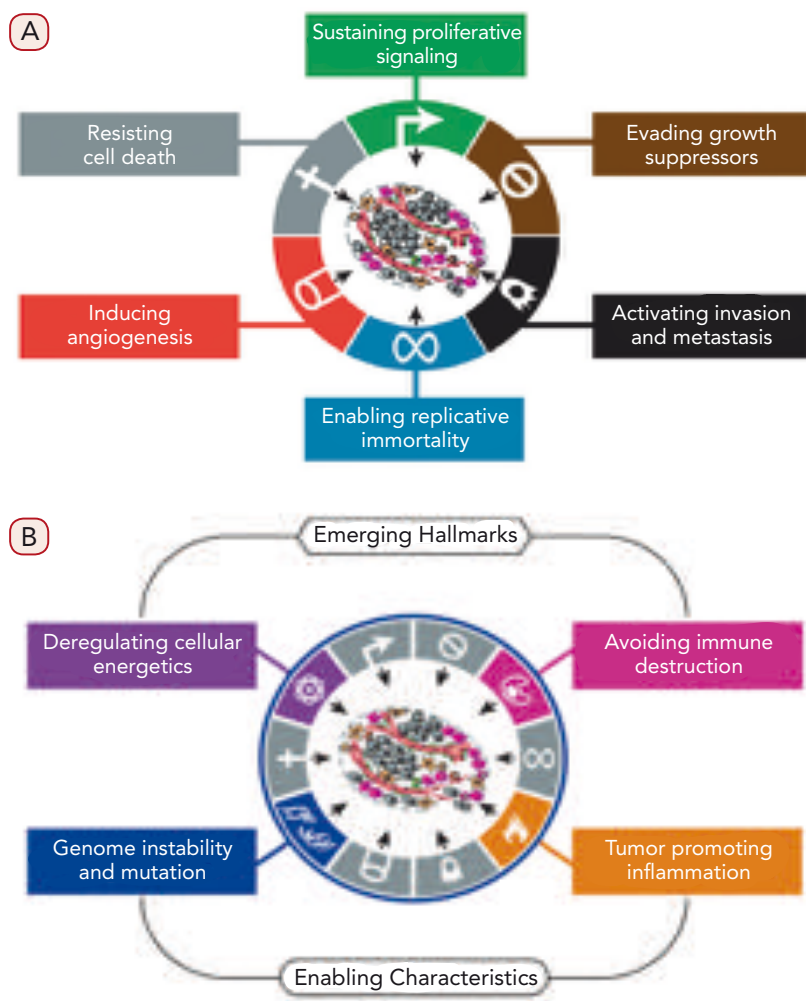
It is well known that the presence of an inflammatory microenvironment can favour tumor formation. Since cancer and inflammation are strictly linked, the hallmarks of cancer originally defined in 2000 have been revisited: inflammation has been included by Hanahan and Weinberg in the “next generation” hallmarks of cancer [1, 2, 3] (• **Figure 1**).

Cancer-related inflammation is caused by an intrinsic pathway generally associated with oncogene activation and by an extrinsic pathway, induced by inflammation and infections. The cellular arm of innate immunity plays an active role in the establishment of cancer-related inflammation and recently it has been demonstrated that also the humoral part is involved [4, 5] (• **Figure 2**).

Macrophages are key players in cancer-related inflammation. They are plastic cells that acquire different phenotypes depending on the tissue where they are located and upon different environmental cues display different effector functions [6, 7]. Tumor-associated macrophages (TAMs) are characterized by a M2-like phenotype, driven by cytokines mainly related to type 2 responses, such as IL-4 and IL-13. They favour tumor growth by affecting not only angiogenesis and immune suppression but also genetic instability, that is a key aspect of cancer [6].

The idea to target macrophages to avoid cancer progression is one of the approaches for immunotherapy. Trabectedin is a natural product derived from a marine tunicate that can be used to target macrophages. It works as an anti-tumor agent *in vivo*, it is approved for clinical use and has an effect on the immune system. Indeed, it has been observed that trabectedin is more toxic on monocytes than tumor cells and it is able to deplete TAMs either in a mouse model of cancer and in humans [8]. Importantly, TAM depletion is the mechanism of action by which trabectedin affects cancer progression [8], representing the first evidence that the targeting TAMs is

• **Figure 1.** Hallmarks of cancer



**A** The six hallmarks of cancer proposed in 2000.

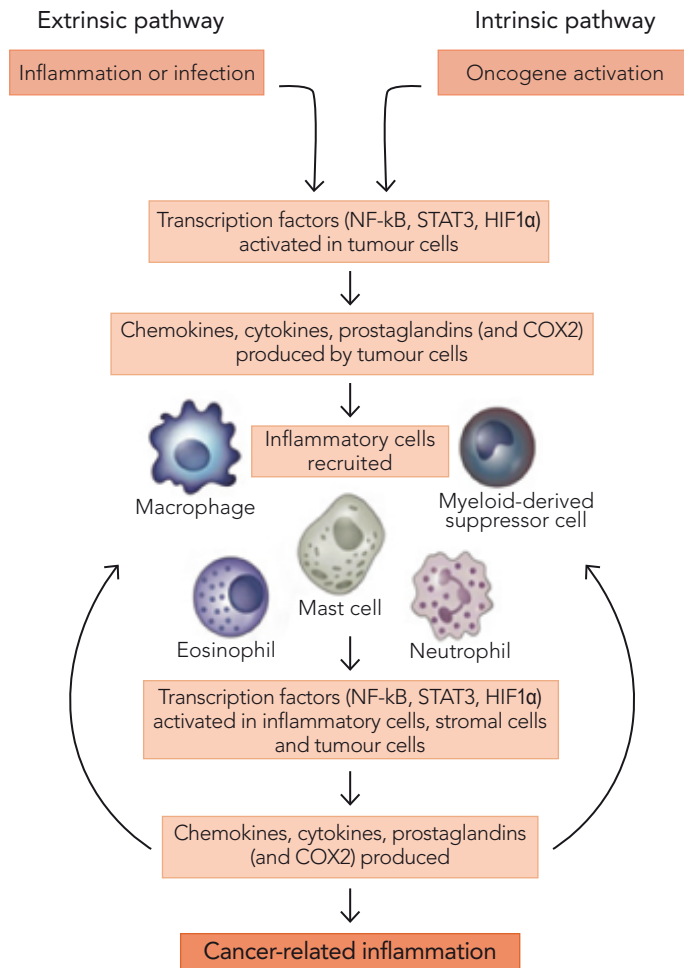
**B** Next generation hallmarks of cancer revisited in 2011.

Source: modified from Hanahan, Weinberg, 2011 [2].

involved in the anti-tumor activity of a clinically approved agent, that could be used in combination with other therapies to improve the clinical outcome.

Among IL-1 inducible genes, some years ago Mantovani *et al.* identified and cloned PTX3 gene, that encodes for a protein aptamer belonging to the pentraxin superfamily [9, 10]. PTX3 is conserved between species and it is involved in microbial

• **Figure 2.** Cancer-related inflammation



Cancer and inflammation are connected by two pathways: the intrinsic pathway and the extrinsic pathway. The intrinsic pathway is activated by genetic events that cause neoplasia. Cells that are transformed in this manner produce inflammatory mediators, thereby generating an inflammatory microenvironment in tumors for which there is no underlying inflammatory condition. By contrast, in the extrinsic pathway, inflammatory or infectious conditions augment the risk of developing cancer at certain anatomical sites. The two pathways converge, resulting in the activation of transcription factors in tumor cells that coordinate the production of inflammatory mediators. These factors recruit and activate various leukocytes, most notably cells of the myelomonocytic lineage. The cytokines activate the same key transcription factors in inflammatory cells, stromal cells and tumor cells, resulting in more inflammatory mediators being produced and a cancer-related inflammatory microenvironment being generated.

Source: modified from Mantovani *et al.*, 2008 [4].

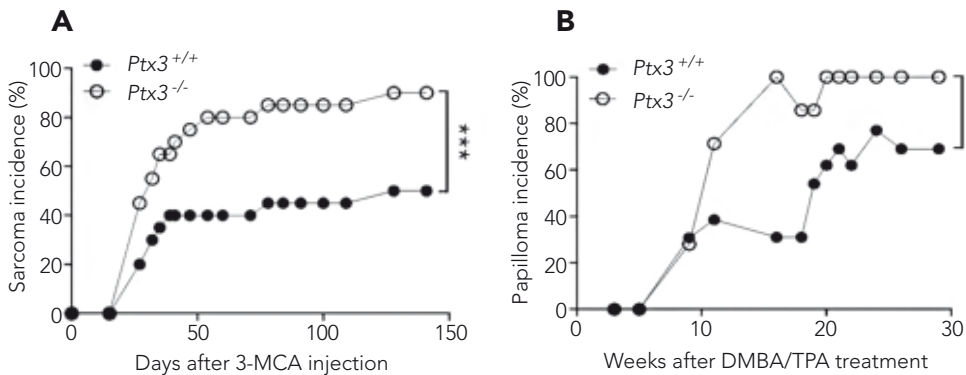
recognition, opsonisation, complement activation and regulation of leukocyte recruitment and inflammation [10, 11]. Furthermore, it has been demonstrated that the absence of PTX3 confers susceptibility to selected microbes (i.e. *A. fumigatus*) [12].

Since PTX3 belong to the humoral compartment of innate immunity and considering the important role of innate immunity in cancer-related inflammation, the contribution of PTX3 to cancer development has been investigated by the use of a spontaneous model of carcinogenesis induced by chemical agents (3-MCA, DMBA-TBA). It has been observed that PTX3 KO mice display higher percentages of sarcoma and papilloma incidence [5] (● Figure 3).

In addition, PTX3 KO tumors produce higher amount of pro-inflammatory cytokines (TNF- $\alpha$ , IL-6, IL-1 $\beta$ ) and chemokines. In particular, high levels of CCL2 nicely correlate with higher monocytes and macrophages infiltration [5]. These results demonstrate that PTX3 is able to regulate cancer-related inflammation.

In order to understand whether this effect is mediated by interactions of PTX3 with P-selectin, the percentage of carcinoma incidence in PTX3 KO mice were compared to P-selectin KO and PTX3 and P-selectin double KO. The absence of P-selectin does not affect carcinoma formation, as the incidence is comparable to the WT. PTX3/P-selectin double KO recapitulate the phenotype of PTX3 KO, suggesting that P-selectin has no role in PTX3-mediated regulation of cancer-related inflammation [5]. In opposite, an increased Complement deposition has been observed in PTX3 KO and it has been demonstrated that the lack of PTX3-mediated recruitment of the negative regulator factor-H is responsible for exacerbate

● **Figure 3.** PTX3 deficiency increases susceptibility to carcinogenesis



Incidence of 3-MCA induced sarcoma (A) and DMBA-TBA skin papilloma (B) in Ptx3<sup>+/+</sup> and Ptx3<sup>-/-</sup>.

Source: modified from Bonavita et al., 2015 [5].

C3 deposition. It suggests that PTX3 could regulate cancer-related inflammation by interacting with the complement. Accordingly, C3 KO mice are protected from tumor formation and totally rescue the phenotype.

These results suggest that Complement actively participates to cancer-related inflammation. Preliminary results obtained in a model of DMBA-mediated carcinogenesis (unpublished data) strongly sustain this evidence. Accordingly, C3 KO mice have a reduced incidence of papilloma compared to controls. Finally, PTX3-deficiency is associated with increased p53 mutations in cancer cell lines and higher DNA-damage response, arguing for a protective role of PTX3 in preventing genetic instability. Furthermore, in several human tumors PTX3 promoter is highly methylated, supporting the idea that the silencing of PTX3 favors tumor progression [5].

In conclusion, PTX3 plays a protective role in tumor development by recruiting factor H that blocks complement-driven inflammation and thus monocyte/macrophage recruitment and polarization towards M2-like phenotype. All together, these results highlight the importance of the humoral part in cancer-related inflammation.

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## Conclusions

The first session of the Forum was opened by Pier Paolo Pandolfi, Director of the Cancer Centre and Cancer Research Institute at Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, whose research has been key to explaining the genetic and molecular mechanisms underlying various types of cancer, particularly prostate cancer. Franco Cavalli, Scientific Director of Oncology Institute of Southern Switzerland (IOSI), renowned for the notable contribution given to the eradication of some types of lymphoma, gave an interesting overview how lymphoma are treated up to date and which therapies could be develop in the coming years. Michael Detmar, ETH, Zurich, moved the attention to lymphatic endothelium and its importance in cancer spreading, by proposing new intriguing imaging technics to study it. Jesús Gil, Head of the Cell Proliferation Group, MRC Clinical Sciences Centre, Imperial College Faculty of Medicine, London, and Andrea Alimonti showed how cellular senescence could be crucial in cancer promotion and how it is possible to affected tumor micro-environment by its manipulation. Johann De Bono, Director of Institute of Cancer Research, London, led clinical trials on abiraterone and transformed prostate cancer treatment.


In the second session of the meeting Douglas Hanahan, one of the founding fathers of modern research, Director of the Swiss Institute for Experimental Cancer Research of EPFL, Lausanne, focused on understanding the mechanisms whereby the tumour and its microenvironment interrelate. Mark Smyth, Medical Research Institute, Brisbane, Australia, focused on NK cells and on the mechanisms by which they can control tumor metastatization. Massimiliano Mazzone, Professor of Translational Oncology, Head of the Lab of Molecular Oncology and Angiogenesis VIB Vesalius Research Center, University of Leuven, showed some molecular mechanisms by which is possible to educate Tumor Associated Macrophages.

Finally, closed the Forum Alberto Mantovani, Scientific Director of Humanitas, Milan, Italy, whose research has contributed to characterise the role of the inflammatory microenvironment in tumour cell development, growth and proliferation, and to the subsequent development of important treatments.

Since discussions were extremely intensive as well as the novelty of the research showed, the conclusions of the meetings were promising and totally satisfying. The take home message was encouraging: go on to study the immune system impact on cancer, cause it could effectively considered as the next generation approach to treat tumors.







Immunotherapy has opened a new era in cancer treatment so much so, that the journal “Science” has put it at the top of the most important scientific breakthroughs of recent years. The revolutionary concept underlying this game-changing treatment approach is harnessing the body’s immune system to combat tumors.

The Forum “Cancer immunology makes it to clinic: how cancer will be treated in the coming years” – organized by IBSA Foundation and attended by around 200 participants – brought together prominent scientists from all over the world who have made significant contributions to this important scientific breakthrough.