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## **To inhibit or to boost the ATP/P2RX7 pathway to fight cancer, that is the question**

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## **Abstract**

Despite new biological insights and recent therapeutic advances, many tumors remain at baseline during treatments. Therefore, there is an urgent need to find new therapeutic strategies to improve the care of patients with solid tumors. P2RX7 receptor (P2XR7), an ATP-gated ion channel characterized by its ability to form large pore within the cell membrane, is described by most of the investigators as a “chef d’orchestre” of the antitumor immune response. The purpose of this review is to detail the recent information concerning different cellular mechanisms linking P2RX7 to hallmarks of cancer and to discuss different progresses in elucidating how activation of the ATP/P2RX7/NLRP3/IL-18 pathway is a very promising approach to fight cancer progression by increasing antitumor immune responses.

## **Introduction**

According to the International Agency for Research on Cancer (IARC), the global burden of cancer reached 19.3 million new cases and 9.6 million deaths in 2020 (gco.iarc.fr). One in five men and one in six women worldwide will develop cancer in their lifetime, and one in eight men and one in 11 women will die from the disease (gco.iarc.fr). Nowadays, the five-year prevalence, corresponding to the total number of people living with cancer within five years of diagnosis, is estimated at 50.55 million.

At the beginning of cancer therapy, tumors were surgically removed, then came the time of chemotherapies - with molecules killing dividing cells – in association or not with radiation therapies. During the last 25 years, new approaches revolutionized clinical cancer care in daily practice. The first one relies on the understanding of oncogene dependent cell autonomous proliferation, leading to clinically relevant targeted therapies based on molecular targets. The second one relies on immune oncology and led to the development of immunotherapeutic strategies designed to turn on/off specific immune checkpoints to promote antitumor immunity. Other orientations for fighting cancer progression are continuously on progress and may open new doors for some therapeutic opportunities [1]. However, despite this new arsenal of therapeutics many patients still do not recover and/or relapse after a variable period of time, therefore testifying for the need of new drug development to fight cancer. In this review, we focus our attention on the P2X7 purinoreceptor (P2RX7), the only receptor being activated by high levels of extracellular adenosine triphosphate (eATP) and discuss recent progress in elucidating how activation of the ATP/P2RX7/NLRP3/IL-18 pathway can be a promising approach to fight cancer diseases by increasing antitumor immunity.

## **Exploring the tumor microenvironment to find new therapeutic strategies**

It is now well documented that vascular networks and supporting cells are required to ensure the expansion of healthy tissues as well as the growth of tumors [2]. Thus, in addition to directly target tumor cells, the non-cancerous cells present in the tumor microenvironment (TME) can also be viewed as relevant targets, even if this domain is now more and more complex [3]. These cells are mesenchymal supporting cells (epithelial, myoepithelial, fibroblasts, adipocytes), cells of the vascular system (endothelial, perivascular cells) and cells of the immune system (lymphoid and myeloid lineages). Altogether, they participate in the development of tumors as nicely illustrated by Egeblad and coauthors [4]. Further, the continuous interplay between tumor cells and normal surrounding cells influences tumor growth. Indeed, it is well established that fibroblasts, the most abundant population within connective-tissue cells, fuel tumor growth [5]. Transforming growth factor beta (TGF- $\beta$ ), a growth hormone secreted by tumor and immune cells, favors the conversion of normal

fibroblasts to carcinoma-associated fibroblasts by feeding an autocrine loop involving TGF- $\beta$  and SDF-1 cytokines, which further lead to the production of tumor promoting chemokines [6, 7] and modulate the composition of immune cells within the TME where they can promote or inhibit tumor growth [8–10]. Is there a common intermediate between all these cells and if so, what could it be?

An important step to answer this question was taken by Geoff Burnstock decades ago when he postulated that ATP is released by most cells and can be considered as an extracellular signaling molecule [11]. This discovery was further sustained by the demonstration that eATP promotes calcium influx in macrophages [12], via the seventh member of P2X purinoreceptor family, formerly identified as the cytolytic P2Z receptor [13], which is described below. Finally, the importance of the ATP/P2RX7 pathway in the field of oncology was buried by the discovery that ATP is present at high levels (millimolar order) in tumor site [14]. Of interest, all mammalian cells express *P2RX7* mRNA and most of the cells express a functional receptor, *i.e.* a receptor expressed at the cell membrane, with a channel and a macropore opening activities [15]. In particular, functional P2RX7 was described in cells that composed the TME, such as fibroblasts [16], endothelial cells [17], dendritic cells [18–20], monocytes/macrophages [21, 22], lymphocytes [23], M- and G-MDSC [24] and neutrophils [25] as illustrated in **Figure 1**. In addition, expression of a functional P2RX7 was found in both solid [26–29] and liquid cancers [30–32].

### **The P2X7 purinoreceptor: A signaling hub underpinning tumor onset**

The P2X7 purinoreceptor (also called P2X7R or P2RX7) belongs to the purinergic receptor family, which is divided into two major families: P2X and P2Y [33]. These receptors have attracted growing interest since the discovery that inflammatory and cancer tissues contain high levels of eATP and adenosine, its immunosuppressive degradation product [14, 34]. Depending on the receptor involved, extracellular purines orchestrate either immune stimulation or immune suppression of host cells, as well as proliferation or cytotoxicity of tumor cells [35].

P2RX7 is an ATP-gated ion channel composed of three protein subunits (encoded by the *P2RX7* gene). Each monomer is composed of two transmembrane domains that are linked by a large extracellular domain made of 285 amino acids; The N and C termini are intracellular, with a very long C-terminus, comprising various moieties such as trafficking and lipid interaction domains and death domain, as nicely illustrated in [36]. This particular feature makes P2RX7 unique, as well as the fact that it is activated by high concentrations of eATP, implicating that it remains active where and when the other P2X receptors showed no current upon agonist application [37].

Activated P2RX7 stimulates the entry of  $\text{Ca}^{2+}$  and  $\text{Na}^+$  but also the efflux of  $\text{K}^+$ . Intracellular  $\text{Ca}^{2+}$  is an ubiquitous second messenger known to control various cellular functions such as cell homeostasis, gene expression, cell survival/death, exo and endocytosis and contraction [38]. The ATP/P2RX7-induced increase of intracellular  $\text{Ca}^{2+}$  levels are highly relevant in multiple cell responses. In macrophages, P2RX7-dependant calcium entry and subsequent activation of the PKC/c-Src/Pyk2/ERK1/2 pathway have been shown to produce reactive oxygen species (ROS) [39], but also lipase activation and blebbing [36]. In the nervous system, P2RX7 activation has been demonstrated to modulate sodium currents and intrinsic neuron excitability [40]. Ion channel function is independent of the long C-terminus, as its deletion or truncation do not alter it [41]. Accordingly, the truncated P2RX7B isoform retains some ATP-induced channel activity and expression of P2RX7B in HEK293 cells increases stimulates growth [42]. However, it is has been shown that domains lying outside of the pore also participate in the regulation of  $\text{Ca}^{2+}$  flux [43].

In addition to increase intracellular  $\text{Ca}^{2+}$  concentrations, P2RX7 activation leads to a decrease in intracellular  $\text{K}^+$  which has been recognized as one of the main activators of NLRP3 inflammasome [44]. Activation of NLRP3 inflammasome is intimately linked to innate immunity being involved in the production of two key potent proinflammatory cytokines, IL-1 $\beta$  and IL-18 which have also been described to be important in tumor onset as they stimulate the production of Interferon gamma (IFN- $\gamma$ ) by immune effector cells (T lymphocytes, Natural Killer) [45, 46]. In addition, activation of NLRP3 inflammasome leads to the formation of pores in the plasma membrane, via Gasdermin D [47], that results ultimately to pyroptotic cell death. Yet, P2RX7 activation by itself is also associated with the formation of a large pore. After decades of research and various hypothesis (mainly pore dilatation and accessory proteins), it is now well admitted that the pore formation is intrinsic of the channel activity [48, 49] and can be regulated by the C-terminus which interacts with phosphatidylglycerols and sphingomyelins of the cell membrane to facilitate cell permeability [50]. Karasawa and co authors also elucidated why the truncated P2RX7 was devoided of pore formation by demonstrating that the C-terminal Cys-rich region, which is deleted in the truncated isoform, counteracts the inhibitory effect of cholesterol, as previously discussed [15, 51].

As already mentioned, the long intracellular C-terminus of P2RX7 is required for most of the signaling pathways induced by the ATP/P2RX7 axis. We will focus here on P2RX7-induced cell responses that are related to hallmarks of cancer (**Figure 2**) as described by Hanahan et al.

### ***P2RX7 and sustained cell proliferation***

Sustained proliferation is a characteristic of tumor cells and is under the control of growth factors that bind receptors with intracellular tyrosine kinase domains which further stimulate

intracellular signaling pathways leading to cell proliferation. ATP, the natural ligand of P2RX7 does not belong to the growth factor family and no tyrosine kinase domains have been described in the C-terminus of P2RX7. However, several reports support the notion that P2RX7 activation by low concentration of ATP, as compared to concentrations inducing cell death, leads to cell proliferation [52–56]. Even in the absence of tyrosine kinase domains, stimulation of the ATP/P2RX7 axis can trigger kinase activation. For instance, Cellular Sarcoma Tyrosine Kinase (c-Src), Mitogen Activated Protein Kinases (ERK1/2, p38, JNK), Phosphoinositide 3-Kinase (PI3Ks)/Protein Kinase B (PKB/Akt) and Protein Kinases C (PKC) have been shown to be activated by P2RX7 [36]. However, no proof of direct interactions exists, and it is likely that kinase activation is mediated via  $Ca^{2+}$  and/or colocalization of P2RX7 and these kinases in lipid rafts. Accordingly, a lipid interaction motif has been described in the C-terminus of P2RX7 (amino acid 574-589) and P2RX7 move toward lipid rafts in response to ATP [57–60]. In addition, the proteomic characterization of P2RX7 protein complex in HEK293 cells expressing the rat P2RX7 isoform identified 11 proteins, some of them being involved in cytoskeletal rearrangement [61], a cell response directly linked to cell proliferation. However, the effect of P2RX7 on sustained cell proliferation is controversial. For instance, in glioma tissue, inhibition of P2RX7 by ShRNA increased the expression of EGF and phosphor-EGF protein [62] and it was reported that reduced expression of P2RX7 may be related to the development of several cancers [15, 63–65].

### ***P2RX7 and growth suppressor evasion***

To hijack programs that negatively regulate cell proliferation is as important as promoting cell proliferation. To our knowledge P2RX7 does not inhibit tumor suppressors such as TP53 or retinoblastoma-associated (RB) proteins. On the contrary, it was reported that the ATP/P2RX7 axis supports apoptosis and necrosis by increasing p53 protein levels in mesangial [66] and human cervical cancer cells [67]. Whether the death domain localized in the C-terminus of P2RX7 [57] is involved in this effect has not been studied. Yet, activation of P2RX7 is associated with mechanisms and or cellular responses leading to growth suppressor evasion since tumors grew faster in *p2rx7<sup>-/-</sup>* mice [68, 69], an effect depending on host immune cells, as discussed below.

### ***P2RX7 and cell death resistance***

Resisting to apoptosis, a programmed cell death viewed as a natural barrier to cancer, is one of the characteristics of tumor cells. Depending on the cellular origin, the ATP/P2RX7 axis induces or inhibits apoptosis. In colorectal cancer cells a pharmacological inhibitor of P2RX7 induced apoptosis [56]. In the MCF7 breast cancer model, decrease of P2RX7 expression with

ShRNA induced cell apoptosis [70]. By contrast, in acute myeloid leukemia, the ATP/P2RX7 axis induced apoptosis [71]. Whether some evidence highlight a relationship between P2RX7-induced signaling and apoptosis, it does not seem that these interactions act as a barrier to cancer. The same conclusion can be drawn for autophagy - an ancestral pathway which maintains cellular homeostasis by degrading long-lived proteins and removing unwanted or unnecessary intracellular components - which represents another cellular response that needs to be circumvented for tumor development. P2RX7-induced signaling pathway has been shown to negatively regulate the autophagic flux through the impairment of lysosomal functions in microglial cells [72]. This pathway, in association with HSP90, was also linked to autophagic cell death in myoblasts and was triggered by large pore formation [73]. In contrast, P2RX7-induced necrosis may be of particular relevance since it allows the release of growth factors which foster proliferation and invasiveness of cancer cells [54], but also immunomodulatory cytokines as discussed below. Finally, it has been shown that tumor cells express a non-conformal receptor (nfP2RX7) which is unable to induce cell death but retains calcium channel activity and therefore could promote tumor growth [74, 75]. The exact nature of this nfP2RX7 still remains an enigma but it may be linked to P2RX7B expression, as discussed in the P2RX7 and genomic instability chapter.

### ***P2RX7 and replicative immortality***

The unlimited replicative property of cancer cells is required to generate macroscopic tumor. P2RX7 could favor cell proliferation (see above), but whether P2RX7 could be linked to replicative immortality is currently unknown.

### ***P2RX7 and angiogenesis***

The formation of new vessels from pre-existing ones, called neo angiogenesis, is required to fuel tumors with oxygen and nutrients. The architecture of the tumor vasculature is abnormal, i.e. leaky, tortuous, fragile, and blind-ended with few or no perivascular cells. Such defectiveness leads to hypoxia and acidity in the TME, further reinvigorating tumor aggression. Therefore, normalization of abnormal tumor vasculature, resulting in enhanced drug delivery, is as important as inhibiting neovascularization. Angiogenesis is under the control of a growth factor called Vascular Endothelial Growth Factor (VEGF) which binds to the VEGF receptor (VEGFR2) to increase endothelial cell proliferation. P2RX7 has been reported to promote VEGF release from human monocytes and rat glioma cells [76, 77]. Tumors obtained after engraftment of human embryonic kidney cells expressing P2RX7 are more angiogenic than tumors obtained with control cells [52]. Mechanistically, it was proposed that the ATP/P2RX7 axis activates the PI3K/AKT and NFκB pathways which further



lead to increased production of VEGF and increased-vessel density [52, 78, 79]. Therefore, in tumor mice models, P2RX7 activation likely stimulates angiogenesis. However, to our knowledge no study in human cancer demonstrates a link between P2RX7 expression and neo angiogenesis.

### ***P2RX7 and invasion and metastasis***

Malignant cells possess the ability to invade surrounding tissues and metastasize. Importantly, the metastatic spread of the primary tumor accounts for over 90% of patient mortality associated with solid cancers [80]. Metastasis is closely associated to the epithelial-mesenchymal transition (EMT), a cellular response being under the control of transcription factors belonging to the Twist and Snail families, which in turn inhibit the expression of epithelial marker (E-cadherin) and increase the expression of mesenchymal markers (vimentin, N-cadherin) [81]. P2RX7 is highly expressed and fully functional in the highly invasive human breast cancer cells MDA-MB-435s; its activation leads to the acquisition of a pro migratory phenotype and enhanced cancer cell invasiveness *in vitro* and *in vivo* [82, 83] thanks to the release of extracellular matrix degrading proteases. In lung cancer cells, the ATP/P2RX7 axis induced an autocrine regulation of TGF- $\beta$ -induced cell migration [84]. Further, P2RX7 effect on cell migration and invasion was observed in response to ATP in pancreatic ductal adenocarcinoma cells [54], but also in the human T47D breast cancer cells and colon cancer cells, where activation of the PI3K/AKT/GSK3 pathway in response to ATP regulates E-cadherin expression and EMT [56, 85]. Whereas many reports highlight the ability of P2RX7 to induce cell invasion and migration in cultured tumor cells, only one study demonstrates its implication in metastasis, considering that metastases correspond to secondary tumors growing at distance of the primary tumor. Using the mammary 4T1 cancer mouse model, it was shown that P2RX7 expression promotes tumor growth and metastasis development, both of them being reduced when mice were treated with specific P2RX7 antagonists [86]. Mechanistically, P2RX7 expression enhances the ECM-degradative activity of invadopodia and triggers the acquisition of a more aggressive phenotype, characterized by activation of Cdc42Rho-GTPase, remodeling of F-actin, elongation of cells and formation of filopodia. Further, a higher expression of P2RX7 has been documented in bronchoalveolar lavage cells of tumors with distant metastasis [87] and in B16 melanoma-induced lung metastasis in mice. However and in contradiction with the above findings, more lung tumors were observed with B16 melanoma cells injected in *p2rx7*<sup>-/-</sup> mice, suggesting that endogenous P2RX7 activity held by host cells restrains B16 migration and invasion [69]. Undoubtedly, additional experiments are required to fully appreciate the role of P2RX7 in invasion and metastasis depending on the cancer origin.

### ***P2RX7 and genomic instability***

Genomic instability of cancer cells favors the selection of subclones demonstrating growth advantage and nowadays study of tumor mutational load is proposed as a sensitive and quantitative clinical marker that can help predict responses to cancer treatment. To our knowledge, P2RX7 was not reported to directly induce DNA mutations. However, several number of missense mutations are present in human *P2RX7* and until now 16 Single Nucleotide Polymorphisms (SNPs) have been characterized in the coding region of human *P2RX7* gene [88]. These mutations affect P2RX7 activity. For instance, the polymorphism in exon 13 at the +1513 position (Glu496Ala substitution, corresponding to SNP rs3751143) has been shown to eradicate the function of this receptor and to be associated with papillary thyroid cancer, hepatocarcinoma and chronic lymphocytic leukemia [89–92]. However, a recent meta-analysis studying the link between rs3751143 and cancer risk minored these results [93]. Nevertheless, in the field of hematological malignancies, P2RX7 and its genetic variants are now considered as biomarkers and potential therapeutic tools [94].

In non-small cell lung cancer P2RX7 expression and the SNP rs3751143 does not impact tumor onset [95], whereas this SNP was associated with reduced metastatic free survival in anthracyclines treated breast cancer [45]. On the contrary, a genetic statistical interaction between P2RX7 (rs3751143, rs208294) and VEGFR-2 (rs2071559, rs11133360) genotypes may identify a population of prostate cancer patients with a better prognosis [96]. In addition to SNPs, the pre-messenger RNA of *P2RX7* is subjected to alternative splicing leading to the formation of 12 *P2RX7* mRNA variants [15, 97, 98]. The function of only four of them, *P2RX7B* with a deletion in the C-terminal domain ( $\Delta$ Ct) leading to a loss of large pore forming function, *P2RX7H* with a deletion in the first transmembrane domain ( $\Delta$ TM1) leading to a loss of ion channel function, *P2RX7J* with a deletion in the second transmembrane domain leading to a negative dominant isoform and the recently described *P2RX7L*, characterized by skipping of exons 7 and 8 leading to deletion of amino acids in the ATP binding site, have been studied in more details [42, 64, 98–100]. The truncated *P2RX7J* was first described to antagonize P2RX7A function in cancer cervical cells, this effect was associated with apoptosis-inhibitory actions [64]. More recently, the *P2RX7B* splice variant has been shown to be differentially upregulated in immune cells of human Non-Small Cell Lung Cancer. This overexpression correlates with a default in the activity of P2RX7 in immune cells and lung tumors expressing *P2RX7B* are less inflamed. Therefore, considering that expression of *P2RX7B* in lung tumors correlated with both an alteration in the P2RX7 function and the lesser infiltrated tumor phenotype (also called “cold” tumor), it was proposed that P2RX7B participates in lung tumor development [101]. A similar conclusion was drawn in patients with AML. In those patients expression levels

of both *P2RX7A* and *B* mRNA are increased and daunorubicin, the main chemotherapeutic for AML, only reduced the expression levels of *P2RX7A*, whereas *P2RX7B* levels remained high, which correlate with cells more prone to survive [102]. One may hypothesize that the heterotrimers composed of *P2RX7A* and *P2RX7B* isoforms may correspond to the nf*P2RX7*, since they shared the same biological activities e.g., no large pore formation and low Ca<sup>2+</sup> channel activity [75].

### ***P2RX7 and reprogramming energy metabolism***

Otto Warburg was the first to describe that cancer cells can reprogram their glucose metabolism, thanks to glycolysis, even in the presence of oxygen [103]. In the heterotopic HEK293 cell system, expression of *P2RX7* leads to the regulation of numerous glycolytic markers conferring to this receptor the power to control cellular energy metabolism [104]. In a human cohort which includes more refined measures of glucose homeostasis, *P2RX7* has been linked to glucose regulation [105]. Accordingly, it was shown that *P2RX7* loss of function in mice produces defective energy homeostasis [106]. To our knowledge, no study demonstrated that *P2RX7* directly regulates energy metabolism of cancer cells. Yet, numerous important studies link *P2RX7* to immunometabolism as discussed below.

### ***P2RX7 and immune responses related to tumor onset***

Beside efficiently fighting infections, the immune system is also programmed to eliminate cancer cells that ultimately evade immune surveillance via checkpoint inhibitor expression and secretion of immunosuppressive factors but also survival and migration factors. [107, 108]. As discussed above, *P2RX7*'s functions have been extensively studied in immune cells. We will focus here on *P2RX7*'s immune populations known to be involved in tumor onset [109].

An efficient anticancer immune response is normally sufficient to kill cancer cells. On one hand, soluble neoantigens expressed by cancer cells are recognized by circulating Antigen Presenting Cells (APC) such as dendritic cells (DCs) and macrophages. Then, APC present the tumor antigens on MHC-I and MHC-II molecules to T cells (CD8+, CD4+) leading to priming and activation of effector T cell responses which traffic to tumor bed, recognize tumor antigen and kill tumor cells. On the other hand, the Natural Killer (NK) cells can directly kill tumor cells by using cytolytic granules and death receptors and by potentiating adaptive immunity through the production of cytokines such as Interferon gamma (IFN- $\gamma$ ) [110]. These antitumor immune responses are blunted by immunosuppressive cells such as regulatory T cells (Treg), and myeloid derived suppressor cells (MDSC) [111].

Both macrophages and DCs from human origin express P2RX7 which is able to modulate cytokine production in response to eATP and LPS [112–115]. The potential of the ATP/P2RX7 axis to enhance antitumor immune responses was suggested by Kroemer's team after the demonstration that eATP attracts DC precursors into the tumor bed, facilitates their permanence in the proximity of dying cells and promotes their differentiation into mature DCs endowed with the capacity of presenting tumor-associated antigens [116]. In addition, P2RX7 activation in macrophages and DCs induces NLRP3 leading to increased production of IL-1 $\beta$  and IL-18. Both pro-tumorigenic and anti-tumorigenic roles of NLRP3 inflammasome have been described in cancers [117]. This double-edged sword effect may depend on the quantity of cytokine produced. For instance, more IL-18 will trigger a more efficient antitumor immune response [118]. Recent studies involving patient with acute myeloid leukemia revealed that *P2RX7* mRNAs (A and B) are upregulated in immune cancer versus immune non cancer cells and AML patients with higher levels of *P2RX7* mRNA show a lower survival rate [102, 119]. Further, engraftment of human myeloid cell lines in immunodeficient mice leads to tumor growth and treatment with specific antagonists of P2RX7 decreases the size of the tumors and increases mice survival rate. The importance of the ATP/P2RX7 axis in leukemic models was further improved by the discovery that P2RX7 is highly expressed in leukemia initiating cells where it promotes AML development by sustaining Ca<sup>2+</sup>-dependent cell signaling [120].

Human lymphocytes also express P2RX7 and demonstrated a growth promoting activity [121, 122]. Of note, at least in mice, P2RX7 expression levels is heterogenous across immune cells. Indeed, it is highly expressed by macrophages, DCs and monocytes while NK cells, CD8<sup>+</sup> and CD4<sup>+</sup>foxp3<sup>-</sup> T cells, neutrophils and eosinophils express very low levels [123]. Yet, it was shown that P2RX7 is required for the establishment, maintenance and functionality of long-lived memory CD8<sup>+</sup> T cells (T<sub>RM</sub>), an effect that depends on P2RX7-dependent metabolic fitness [124]. Interestingly, in a melanoma mice model, T<sub>RM</sub> can actively suppress cancer progression [125]. P2RX7 is also expressed by human Tregs. It was shown that esophageal carcinoma from patients expressing the loss-of-function allele of *P2RX7* are more infiltrated with Tregs than tumors from patients bearing the WT allele of *P2RX7* [126]. The same tendency was observed in the colitis associated cancer mice model and melanoma models, where *p2rx7*<sup>-/-</sup> mice developed more tumors and tumors were more infiltrated with Tregs than tumors grown in WT mice [68, 127]. Such results could be explained by the fact that P2RX7 controls Treg homeostasis and in absence of P2RX7, Treg death is decreased [128, 129]. Finally, whereas the level of P2RX7 expression in human MDSCs has not yet been reported, it was shown in the neuroblastoma mice model that the ATP/P2RX7 axis increases immunosuppressive functions of MDSCs [130]. In conclusion, while it is indisputable that P2RX7 modulates immune cell functions, the contradicting results found in the literature make

it is difficult to predict whether P2RX7, by itself, impinges or enhances antitumor immune responses.

### **Should we target P2RX7 in cancer biology?**

This is indeed THE QUESTION, and as evidenced in previous chapters, this is a puzzling question (**Figure 3**). Should we inhibit P2RX7 in order to blunt its proliferative effect on cancer cells? However, this scenario may be dangerous since it may also inhibit antitumor immune responses sustained by P2RX7? Should we activate P2RX7 in order to boost its cytotoxic effect on tumor cells? But is P2RX7 really cytotoxic on tumor cells, since we know that many tumors express a nfP2RX7? If we activate P2RX7, would it really enhance antitumor immune responses or would it boost the function of immunosuppressive cells that are recruited in the TME?

Since the notion of immunosurveillance emerges only recently in the field of cancer biology [131] and because big pharma were more prone to develop inhibitors, especially P2RX7 antagonists that may treat inflammatory disorders, numerous small pharmacological inhibitors and biologics were tested in preclinical and clinical studies as reviewed in [65]. In brief, most of the preclinical studies were based on transplantation in immunodeficient or syngeneic immunocompetent mice of various tumor cell lines (e.g., HEK293, neuroblastoma, colon carcinoma, melanoma, glioma, osteosarcoma and myeloid leukemia) endowed with endogenous or exogenous expression of P2RX7, where tumor growth was significantly reduced by treatment with AZ10606120, A438079 or A740003, three well described antagonists of P2RX7 [52, 54, 55, 69, 127, 132]. However, an acceleration of tumor growth was also described when tumor cell lines were transplanted in *p2rx7* KO mice or with chemically -induced in situ colon tumors [68, 69]. These results highlight the importance of P2RX7 expressing host cells in tumor onset and suggest that the levels of expression and functionality of P2RX7 in tumor cells is a critical issue that have to be considered before the use of antagonists to treat tumor. As far as we know, no clear benefits to patients have been reported so far and this lack of effect may be related to the fact that inflammatory tissues contain high levels of ATP, a condition that does not favor the binding of antagonist to the allosteric binding site [133]. The use of biologics may be an alternative. In particular, application of an antibody that specifically recognizes the E200 epitope of nfP2RX7 appears to be promising for the local treatment of the primary basal cell carcinoma lesion [134].

We hypothesized that increasing P2RX7's dependent antitumor immune response by enhancing its activation would inhibit tumor growth. We developed positive allosteric modulators (PAMs) of P2RX7, i.e. molecules that enhances P2RX7 activity in the presence of ATP. HEI3090 was selected as the most potent PAM that enhances P2RX7's activities only in

the presence of ATP and showed that it efficiently inhibits tumor growth. HEI3090 enhances antitumor immune responses by boosting the ATP/P2RX7/NLRP3/IL-18 pathway in DCs, sensitizes tumor to anti PD-1 immunotherapy and triggers long lasting antitumor immune responses [135]. Our bet looks like a successful story, which is not so surprising considering that anti CD39 antibody was recently shown to promote the eATP/P2RX7/NLRP3/IL18-driven antitumor responses [123]. In addition, activation of this pathway with anti CD39 monoclonal antibody in myeloid cells activates effector function of NK cells and suppresses experimental and spontaneous metastases in a tumor of different origins [136], therefore bringing new hopes for the treatment of cancers. However, more work is needed to understand which immune cells should be targeted to kill tumor cells in order to reconcile data showing a benefit to activate the ATP/P2RX7 axis in specific immune cells (macrophages, DCs, NK) and data showing that the lack of P2RX7 in tumor-specific T cells reduced tumor growth [137]. Further, we need to understand why tumor fate is different in *p2rx7* null mice and mice treated with an antagonist of P2RX7 [127].

## **Perspectives**

Undoubtedly, targeting P2RX7 to treat cancer is a promising strategy and recent data from several laboratories highlighted the capacity of the ATP/P2RX7/NLRP3/IL-18 axis to activate antitumor immune responses. However, pivotal questions remain to be answered. Among them, how do the new pharmacological positive modulator of P2RX7 (HEI3090) enhance the receptor activity? Does it bind to the receptor itself and if so, where? Is HEI3090 active on the nP2RX7? And if so, which and how, P2RX7-dependent biological responses are going to be regulated? Would it be interesting to combine HEI3090 with the new anti CD39 antibody in order to maximize the activation of the ATP/P2RX7/NLRP3/IL-18 axis and the consecutive antitumor immune response? Would the other non-nucleotide agonists of P2RX7 (i.e. ginsenoside, LL37 [138, 139]) will also boost the antitumor immune responses supported by the ATP/P2RX7 axis? and if so, could we combine them with HEI3090 and immunotherapies? What would be the effect of an agonist of P2RX7 on inflammatory pain and IL-1 $\beta$ -induced cachexia, knowing that P2RX7 antagonists not only efficiently inhibit the production of IL-1 $\beta$  but also reduced cancer-induced bone pain [140, 141]? In this perspective, the use of HEI3090 may be of particular interest, since we showed in transplantable and oncogene-driven tumor models that it increases seric IL-18 levels with no effect on IL-1 $\beta$ . We believe that these points will be resolved in a near future, and in addition, we are strongly convinced that their resolution will bring new innovative strategies to fight cancers.

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**Informed consent:** Not applicable, no studies with human

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## Figure legends

### **Figure 1 : P2RX7 expression in eukaryotic cells**

Illustration representing non-immune and immune cells expressing P2RX7 and the main ATP/P2RX7-induced cellular responses.

### **Figure 2 : P2RX7-induced cell responses related to hallmarks of cancer**

This illustration summarized the relationship between P2RX7 and cancer's hallmarks. Both general functions and the mode of action of P2RX7 on these general functions (text in the box) are shown.

### **Figure 3 : The yin and yang of P2RX7's modulators**

Dying damage cells and activated immune cells (in red) produce micromolar concentration of ATP leading to P2RX7 activation in TME and inflammatory tissues. Whereas cancer cells are always considered as foes, immune cells can be either friends or foes depending on their ability to modulate anti- or pro-tumor responses. P2RX7 being expressed by both cancer cells and immune cells, two treatment scenarios are possible: the use of antagonists in order to blunt cancer cell proliferation and invasion with the risk of blocking antitumor responses (red square) or the use of agonists aiming at increasing P2RX7-induced cell death and boosting antitumor immune responses (green square).



**Figure 1**

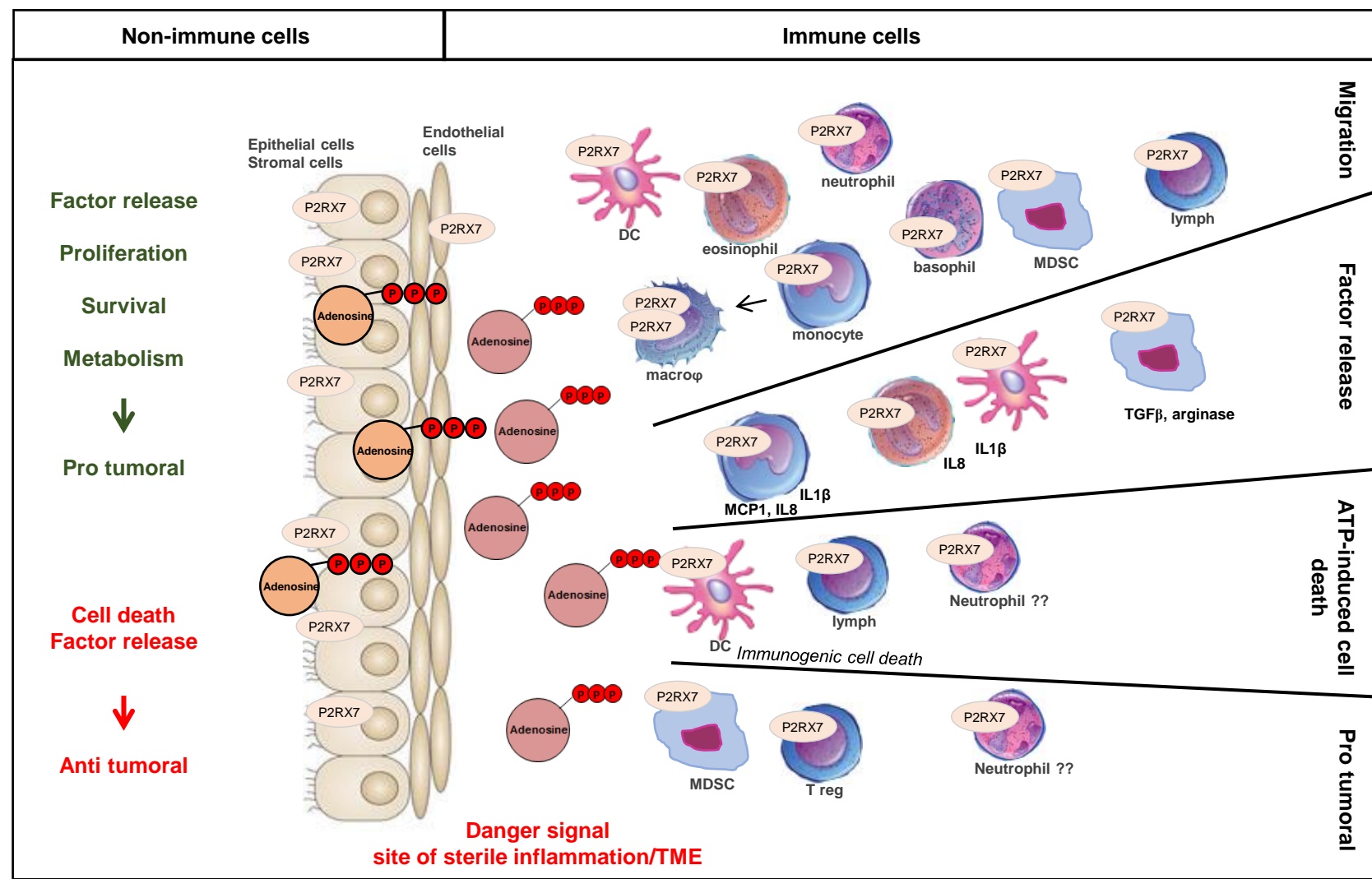


Figure 2

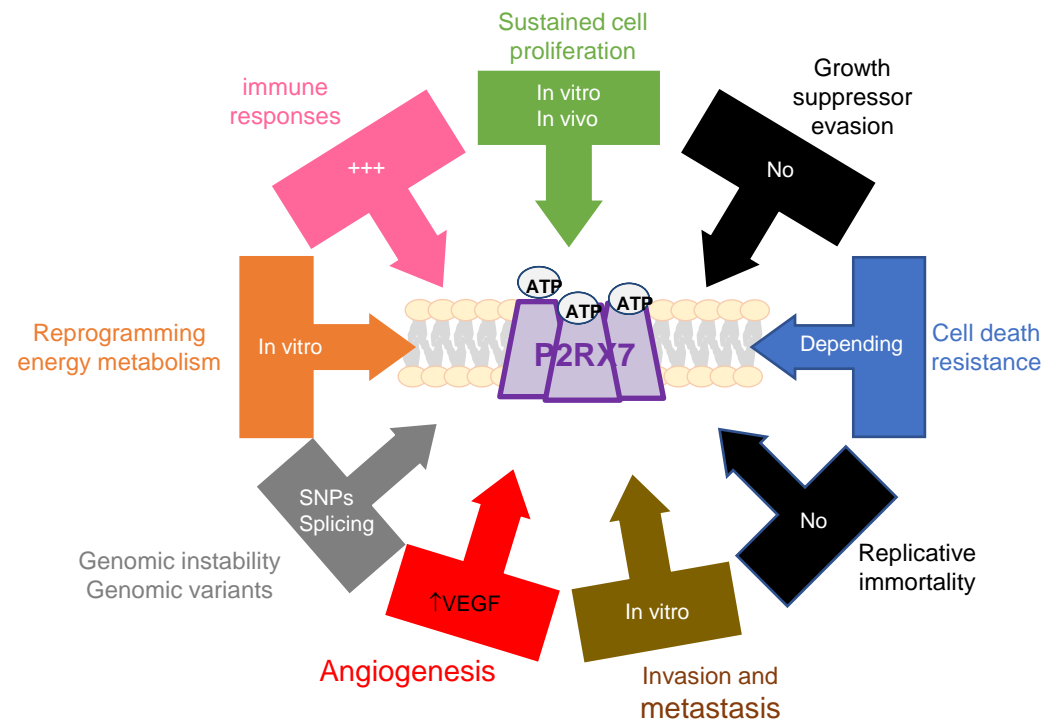


Figure 3

