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Reference:

Jacobs Julie, Deschoolmeester Vanessa, Zwaenepoel Karen, Rolfo Christian, Silence K., Rottey F., Lardon Filip, Smits Evelien, Pauwels Patrick.- CD70 : an emerging target in cancer immunotherapy

Pharmacology and therapeutics - ISSN 0163-7258 - 155(2015), p. 1-10

Full text (Publishers DOI): <http://dx.doi.org/doi:10.1016/j.pharmthera.2015.07.007>

To cite this reference: <http://hdl.handle.net/10067/1285100151162165141>

CD70: an emerging target in cancer immunotherapy

Jacobs J^{1,2}, Deschoolmeester V^{1,2}, Zwaenepoel K², Rolfo C^{3,4}, Silence K⁵, Rottey S⁶, Lardon F¹, Smits E^{1,7,*}, Pauwels P^{1,2,*}

Authors' affiliations: ¹Center for Oncological Research, University of Antwerp, Wilrijk, Belgium;

²Department of Pathology, Antwerp University Hospital, Edegem, Belgium; ³Department of Oncology,

Antwerp University Hospital, Edegem, Belgium; ⁴Phase 1-Early Clinical Trials Unit, Antwerp University

Hospital, Edegem, Belgium; ⁵arGEN-X BVBA, Ghent, Belgium; ⁶Department of Medical oncology, Ghent

University Hospital, Ghent, Belgium; ⁷Laboratory of Experimental Hematology (LEH), Vaccine and

Infectious Disease Institute, University of Antwerp, Edegem, Belgium; * Co-senior authors

Corresponding author: Julie Jacobs, Center for Oncological Research, University of Antwerp, Wilrijk, Belgium. Phone: 00323/2652533; E-mail: julie.jacobs@uantwerpen.be.

Abstract

Over the last decades, advances in the knowledge of immunology have led to the identification of immune checkpoints, reinvigorating cancer immunotherapy. Although normally restricted to activated T and B cells, constitutive expression of CD70 in tumor cells has been described. Moreover, CD70 is implicated in tumor cell and regulatory T cell survival through interaction with its ligand, CD27. In this review, we summarize the targetable expression patterns of CD70 in a wide range of malignancies and the promising mechanism of anti-CD70 therapy in stimulating the anti-tumor immune response. In addition, we will discuss clinical data and future combination strategies.

Keywords

CD70, CD27, Cancer immunotherapy, Combination therapy

Abbreviations

ADCC, Antibody-dependent cellular cytotoxicity; ADCP, Antibody-dependent cellular phagocytosis; CDC, Complement-dependent cellular cytotoxicity; CTLA-4, Cytotoxic T lymphocyte antigen-4; FOXP3, Forkhead box P3; HIV, Human immunodeficiency virus; IHC, Immunohistochemistry; IL, interleukin; iTregs, induced Tregs; LPC, Lymphoplasmacytic cells; MMP, Matrix metalloproteinase; NHL, non-hodgkin lymphoma; NK, Natural killer; NSCLC, Non-small cell lung cancer; nTregs, naturally occurring Tregs; PD(L)-1, Programmed death (ligand); RCC, Renal cell carcinoma; sCD27, Soluble CD27; TCL, T cell lymphoma; TCR, T cell receptor; TGF- β , Tumor growth factor- β ; TIL, Tumor-infiltrating lymphocyte; TNF, Tumor necrosis factor; TRAF,

Tumor receptor-associated factor; Tregs, Regulatory T cells; WM, Waldenström macroglobulinemia.

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1. Introduction

The mutual and interdependent interaction between the cancer cells and their microenvironment is increasingly considered a crucial domain of investigation in cancer research. In this regard, immunotherapy represents a promising therapeutic modality in oncology, as evidenced by its election as Science's Cancer Breakthrough 2013 (Couzin-Frankel, 2013). Recently, much interest has been generated by the clinical results associated with inhibition of immune checkpoint proteins by antibodies directed against cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death (ligand) -1 (PD-1/PDL-1) (Roflo et al., 2014). In this review, we will focus on the CD70-CD27 signaling pathway, emerging as an interesting new field of study to enhance anti-tumoral immune responses. Indeed, the discovery of CD70 expression on multiple tumor types of hematological origin and also on several types of solid tumors makes this molecule an attractive target for antibody-based immunotherapy.

CD70 belongs to the tumor necrosis factor (TNF) superfamily of molecules, consisting of over 20 membrane-bound and secreted protein ligands. This protein is a type II transmembrane glycoprotein, comprised of 193 amino acids with a molecular mass of 50kDa. Sequence homology with other TNF superfamily members predicted the appearance of CD70 as a homotrimer leading to the understanding that interaction with CD27, its unique receptor, may involve three CD27 homodimers (Boursalian et al., 2009). Upon interaction with CD70, cytoplasmic residues of CD27 are bound to TNF receptor-associated factors (TRAFs), such as TRAF2 and TRAF5, thereby activating NF κ B and c-Jun kinase pathways, leading to proliferation, survival and differentiation (Boursalian et al., 2009). Additionally, a role of CD27 in caspase-mediated apoptosis is suggested via the receptor-associated death domain-containing adaptor protein Siva (see figure 1) (Prasad et al., 1997). As new insights into the CD70-CD27 pathway have been gained over the last decade, we will discuss novel approaches to target this pathway in human malignancies.

2. CD70-CD27 physiology

The TNF receptor superfamily member CD27 is a tightly regulated costimulatory molecule, activated through its unique ligand CD70, enabling activation of innate and adaptive immunity. In humans, expression of CD27 is detected on thymocytes and naïve T cells, upregulated upon T cell activation and diminishing after effector T cell differentiation (Hintzen et al., 1994). In addition, CD27 is also found on central memory T cells, residing in secondary lymphoid organs (6). Despite these expression patterns, CD27-deficient mice have normal T cell development in the thymus and similar numbers of naïve T cells in secondary lymphoid organs as opposed to wild-type controls. However, depletions in the effector T cell pool and impeded memory T cells are observed (Hendriks et al., 2000; Hendriks et al., 2003). These studies indicate that CD27 triggering is neither required nor sufficient to induce effector T cell formation, but contributes to the formation of the effector T cell pool by efficient priming of T cells and the subsequent promotion of T cell survival (Nolte et al., 2009). The role of the CD70/CD27 axis in the priming of T cells was also demonstrated in a variety of immunization and infection models (Matter et al., 2005; Nolte et al., 2009). CD27-CD70 interactions were shown to induce proliferation and cytokine production by both CD4⁺ and CD8⁺ T cells and promote development of cytotoxic T cell responses (Lens et al., 1998). Nonetheless, CD70 knock out mice show normal CD4 T cell responses and memory CD8 T cell generation after lymphocytic choriomeningitis virus infection (Munitic et al., 2013). Instead, persistent signaling of CD27 upon lymphocytic choriomeningitis virus infection induces immunopathology and suppression of neutralizing antibodies, indicating CD27 activity should be carefully controlled in order to prevent collateral damage (Matter et al., 2006). This is established by the strict control of CD70 expression under physiological conditions: transiently upregulated on antigen-activated T and B cells, waning following the removal of the antigenic stimulus and mainly detected in primed effector lymphocytes (Borst et al., 2005). In mature dendritic cells, CD70 expression is upregulated upon triggering of CD40 or Toll-like receptors (Boursalian et al., 2009). Thereby an intrinsic

pool of CD70 is transported towards the immunological synapse with MHC-II molecules, ensuring optimal T cell stimulation (Keller et al., 2007; Kuka et al., 2013). Besides its effect on T cell development, CD27 is upregulated on B cells through antigen receptor triggering and is maintained after activation, making it a typical marker for memory B cells (Jacquot et al., 1997). Despite the fact that triggering of the CD70/CD27 axis stimulates immunoglobulin production by the promotion of plasma cell differentiation, CD27 is not absolutely required for adequate B cell responses, since deficiency does not affect isotype switching, somatic hypermutation or antibody production (Agematsu et al., 1999; Xiao et al., 2004). Moreover, in mice that constitutively express CD70 on B cells, T cells or dendritic cells, a demise of B cells in the bone marrow and secondary lymphoid organs is seen due to the chronic activation of CD27 (Nolte et al., 2009). In these mouse models, the expression of CD70 by B cells even resulted in exhaustion of the naïve T cell pool, depletion of T cells from lymph nodes and death from opportunistic infection (Tesselaar et al., 2003). As for B cells and T cells, CD27 is highly regulated on natural killer (NK) cells, the key mediators of innate immune defense mechanism. Evidence indicates that NK cells upregulate CD27 in their final developmental stage before leaving the bone marrow. However, on circulating NK cells CD27 is absent, implicating that CD27 is turned off when these cells acquire their highest effector cell potential (Vossen et al., 2008). The impact of the CD70-CD27 pathway on human NK cells is still largely unknown since CD27-deficient mice show normal amounts of NK cells with adequate function properties (Vossen et al., 2008). However, in mice that constitutively express CD70 on B cells, continuous CD70-CD27 interactions result in a severe reduction of NK cell numbers (De Colvenaer et al., 2010). Overall, CD70-CD27 interaction is crucial for the regulation of the cellular immune response leading either to improved T cell function or T cell dysfunction, whereby timing, context, and intensity of these costimulatory signals determine the functional consequence of their activity (Nolte et al., 2009). In this regard overexpression of CD70 can be observed in different auto-immune diseases, such as rheumatoid and psoriatic arthritis and lupus (Boursalian et al., 2009; Han et al., 2005; Lee et al., 2007; Oelke et al., 2004). In the next

sections we will discuss the role of the CD70/CD27 axis in tumor biology as it is becoming clear that CD70 can also serve as a target for cancer immunotherapy.

3. CD70-CD27 in tumor biology

- **Expression patterns**

In addition to the transient expression of CD70 on antigen-activated T and B cells and mature dendritic cells, recently constitutive expression of CD70 has also been found on a unique population of antigen presenting cells, exclusively localized in the gut lamina propria (Keller et al., 2007; Laouar et al., 2005; Nolte et al., 2009). Beyond this, CD70 is generally absent in non-lymphoid normal tissues (Jacobs et al., 2014). Contrarily, constitutive CD70 expression on many types of hematological malignancies and solid carcinomas has been observed. High rates of CD70 expression are particularly found in lymphomas, renal cell carcinoma (RCC), nasopharyngeal carcinoma, as well as in Epstein-Barr virus-induced carcinomas (Table 1) (Agathangelou et al., 1995; Boursalian et al., 2009; Israel et al., 2005). In B cell lymphoma, RCC and breast cancer, CD70 expression is associated with poor prognosis (Bertrand et al., 2013; Jilaveanu et al., 2012; Petrau et al., 2014). Interestingly, next to its expression on primary tumor-biopsies, stable CD70 expression is also found on patient-derived metastatic tissue in up to 100% of cases (Jacobs et al., 2015; Law et al., 2006). In literature, different methods to detect CD70 expression have been described, including real-time PCR, western blot and immunohistochemistry (IHC) (Diegmann et al., 2005). To date, IHC remains the most frequently used method to determine CD70 expression due to its simple and rapid detection of protein expression. However, in literature, discrepancies in the percentage of CD70-positive cases are seen within identical tumor types. As shown in Table 1, these dissimilarities could be caused by different antibodies and cut-off values which show the need for one uniform and validated methodology. In this regard, testing the methylation status of the CD70 promoter region could be an interesting option. Indeed, constitutive expression of CD70 in patients with large B cell lymphoma, as well as auto-immune

diseases, has been associated with demethylation of the CD70 promoter region (Bertrand et al., 2013; Oelke et al., 2004; Zhou, Qiu, et al., 2011). Likewise, epigenetic aberrations of the CD70 promoter region in breast cancer cells have also been demonstrated to affect CD70 expression (Petrau et al., 2014; S. E. Yu et al., 2010). However, further research is mandatory to unravel whether de-methylation of the CD70 promoter could be an alternative for CD70 IHC.

In addition to the constitutive expression of CD70, many of the hematopoietic lineage tumors also express CD27, which might implicate a possible role for CD27-CD70 interactions in the regulation of tumor cell expansion and survival (Goto et al., 2012; Lens et al., 1999). Nilsson A. et al. found such co-expression on a high proportion of leukemic cells in acute lymphoblastic lymphoma samples (Nilsson et al., 2005). Moreover, using a CD70 blocking antibody, proliferation of the leukemic stem cells could be reduced substantially. Likewise, the coordinated expression of CD70 and CD27 has been suggested to stimulate activation and proliferation of Sézary cells, a variant of cutaneous T cell lymphoma (van Doorn et al., 2004).

Contrarily to its presence in hematological malignancies, CD27-expressing tumor cells in solid malignancies have not been demonstrated to date (Agathangelou et al., 1995; Hishima et al., 2000). Nonetheless, in the tumor microenvironment persistent CD27 signaling can occur through the expression of CD27 on tumor-infiltrating lymphocytes (TILs) which have been shown to communicate with CD70⁺ carcinoma cells (Agathangelou et al., 1995). The importance of tumor and T-cell interaction through the CD70/CD27 axis will be discussed below.

Upon binding of CD70 to CD27, soluble CD27 (sCD27), the extracellular domain of membrane-bound CD27, is cleaved from the cell surface by metalloproteinases (Hintzen et al., 1991; Kato et al., 2007; Loenen et al., 1992). This 32kDa protein has been detected by enzyme-linked immunosorbent assay in serum, plasma and urine samples from healthy individuals, and at increased levels in hematological

malignancies and auto-immune diseases (Font et al., 1996; Goto et al., 2012). In addition, measuring sCD27 levels in healthy individuals as opposed to patients infected with human immunodeficiency virus (HIV) demonstrated elevated serum sCD27 levels in the HIV⁺ subset. Moreover, sCD27 levels were particularly elevated in HIV⁺ patients who developed AIDS-associated non-Hodgkin lymphoma (NHL) (Widney et al., 1999). The soluble form of CD27 has also been linked to poor outcome in various hematological malignancies, such as Waldenström's macroglobulinemia (WM), an indolent B cell malignancy characterized by bone marrow infiltration with lymphoplasmacytic cells (LPCs) (Ho et al., 2008). Ho et al. showed LPC-mediated secretion of sCD27, with statistically significant elevated levels in WM patients in relation to healthy donors. This study also demonstrated the utility of sCD27 as a surrogate marker of disease burden (Ho et al., 2008). Moreover, it was demonstrated that sCD27 could induce upregulation of TNF family ligands on mast cells, providing survival signals to the tumor cells. Additional data indicated a possible role of matrix metalloproteinase-8 (MMP-8) in sCD27 release by cleavage of CD27, since higher transcription levels of this proteinase were found in WM cells and MMP-8 inhibitors resulted in the blocking of sCD27 release (Zhou, Liu, et al., 2011). Elevated serum concentrations of sCD27 were also found in patients with acute lymphoblastic leukemia, chronic lymphocytic leukemia and large B cell lymphoma, predicting poor outcome in the latter and correlating with CD27 expression on lymphoma cells (Goto et al., 2012; Kato et al., 2007; Nilsson et al., 2005; Tadmor & Polliack, 2012). Contrarily to its correlation with outcome in hematological malignancies, no effect on overall survival was found in patients with prostate cancer. Moreover, these patients presented a lower pool of serum sCD27 compared to healthy donors (Huang et al., 2013). Notably, sCD27 did show potential as a prognostic marker in non-small cell lung cancer whereby elevated sCD27 levels correlated with reduced overall survival and progression free survival (Jacobs et al., 2015). In order to evaluate the potential use of sCD27 as a diagnostic biomarker for prognosis more research on a wide range of human malignancies is mandatory.

- **Signaling in the tumor-microenvironment**

Tumor cells are able to evade detection and elimination by the host immune system through distinct mechanisms of action. As a result, lymphocytes either seem to be absent in the tumor-microenvironment or incapable of controlling or rejecting the tumor completely (Rabinovich et al., 2007). Inhibition of T cell function can be triggered by tumor-derived inhibitory cytokines, such as tumor growth factor- β (TGF- β). Furthermore, an immunosuppressive environment can be created through downregulation of NK cell activators, reduced expression of class I MHC proteins by the tumor cells, T cell anergy or activation of immunosuppressive regulatory T-lymphocytes (Tregs) (Rolfo et al., 2014). One interesting player in this regulation of immune escape is the CD70-CD27 signaling pathway, acting through the expression of CD27 on regulatory T cells.

Naturally occurring CD4⁺CD25⁺ Tregs (nTreg), arising in the thymus, have been shown to impede the anti-tumor response in different murine models (P. Yu et al., 2005). In addition, Tregs can be induced (iTreg) outside the thymus from CD4⁺CD25⁻ naïve cells upon T cell receptor (TCR) stimulation, or stimulation by Interleukin-2 (IL-2) and TGF- β . Thereby, the expression of transcriptional factor forkhead box P3 (Foxp3) has been shown to be crucial in the development and immunosuppressive function of Tregs (Hori et al., 2003). In biopsy specimens from patients with NHL, Yang et al. demonstrated significant amounts of intratumoral iTregs, inhibiting cytokine production of CD4⁺ and CD8⁺ T cells (Yang et al., 2006). Interestingly, increasing amounts of iTregs were particularly seen in the presence of CD70-expressing malignant cells. Moreover, blockade of the CD70-CD27 pathway abrogated the induction of Foxp3 expression in intratumoral CD4⁺CD25⁻ T cells (Yang et al., 2007). Likewise, chronic lymphocytic leukemia cells, carrying high CD70 surface expression, were shown capable of inducing Tregs in a CD70 dependent manner (Jak et al., 2009). This was also seen in solid tumors, where Claus et al. demonstrated that CD70-CD27 interactions augmented the frequency of Tregs, reduced tumor-specific T cell responses and promoted tumor growth in solid tumor-bearing mice (Claus et al., 2012). Furthermore, CD27 was

responsible for a decrease in nTreg apoptosis and the production of IL-2, a key player in Treg survival (Claus et al., 2012; Coquet et al., 2013; Riether et al., 2012). In lung tissue, expression of CD27 on CD4⁺ T cells was shown to differentiate between Tregs and effector T cells. Thereby, CD27 correlated with the expression of Foxp3 in Tregs and their immunosuppressive activity (Mack et al., 2009). Only recently, CD70 was suggested as an inducer of Tregs in non-small cell lung cancer (NSCLC). Indeed, increased Foxp3 expression and higher CD4/CD8 ratios were found, surrounding CD70⁺ tumor cells in surgically resected specimens (Jacobs et al., 2015). In addition to its effect on Treg survival, another mechanism of immune escape in the tumor-microenvironment has been attributed to the CD70-CD27 pathway leading to apoptosis in lymphocytes. This ability of CD70-expressing tumor cells to induce immunosuppression through tumor-induced apoptosis of T-lymphocytes was demonstrated in RCC, glioma and glioblastoma cells (Chahlavi et al., 2005; Diegmann et al., 2006; Wischhusen et al., 2002). Thereby, the apoptotic protein Siva, binding the cytoplasmic tail of CD27, was assumed to mediate apoptosis through caspase activation (Diegmann et al., 2006; Prasad et al., 1997). Notably, this induction of apoptosis could be reduced by anti-CD70 antibodies (Chahlavi et al., 2005). A final mode of action why cancer cells hijack CD70 to escape immune surveillance is the induction of T cell exhaustion. In CD70⁺ RCC, Wang et al. demonstrated a significant phenotype shift in TILs towards a much more differentiated profile than seen in CD70⁻ melanoma, leading to the failure of these lymphocytes to destroy the tumor. Thereby, tumor-expressed CD70 was suggested as the inducer of this proliferative exhaustion in TILs (Q. J. Wang et al., 2012). Another role of the pathway in T-cell exhaustion was shown in B cell NHL. Yang et al. phenotypically characterized intratumoral T cells, isolated from specimens of follicular lymphoma patients. Thereby, CD70⁺ T cells appeared to express high levels of CD45RO, a marker of memory T cells, as well as PD-1 and TIM-3. Furthermore, these CD70⁺ T cells exhibited an exhausted phenotype, similar to exhaustion driven by TGF- β . More importantly, by blocking CD70 they were able to improve the viability of CD4⁺ T cells treated with TGF- β (Yang et al., 2014).

In summary, the constitutive expression of CD70 by tumor cells can facilitate evasion of the immune system by three important mechanisms: increasing the amount of suppressive Tregs, induction of T cell apoptosis and skewing T cells towards T cell exhaustion (Figure 2).

4. Targeting CD70

- **Clinical advances**

With respect to CD70 targeting in cancer, three concepts are of particular relevance (Boursalian et al., 2009). First, the constitutive overexpression of CD70 on tumor cells and its absence on normal tissue can be exploited by applying antibody-dependent cellular cytotoxicity (ADCC)-inducing antibodies or antibody-drug conjugates (ADC) to the malignant cells. Secondly, blocking CD70 can abolish its immune inhibitory effects in the tumor-microenvironment. Thirdly, in hematological malignancies, anti-CD70 therapy can also lead to blocking of survival and proliferation signals through the co-expression of CD27 and CD70 on malignant cells. These promising options have led to the initiation of several clinical studies to determine the safety and efficacy of anti-CD70 therapy. Currently, three CD70-blocking antibodies are being tested in these clinical trials, SGN-CD70A, AMG 172 and ARGX-110, which will be discussed below (Table 2).

SGN-75 and SGN-CD70A

SGN-75 is a CD70-blocking IgG1 ADC, releasing its cell-killing agent upon internalization into CD70-expressing tumor cells (Ryan et al., 2010). In a first study by Seattle Genetics, SGN-75 was tested in patients with relapsed or refractory CD70⁺ NHL or metastatic RCC (NCT01015911). Patients were treated intravenously with escalation doses from 0.3 to 4.5mg/kg every 7 or 21 days (Tannir et al., 2014). Among the 19 NHL patients and 39 RCC patients enrolled in the study, three objective responses (1 mantle cell lymphoma patient achieved complete response; 2 RCC patients with partial response) were shown.

Interestingly, all 3 patients with objective responses had a high uniformity of CD70 expression with 95% of tumor cells staining positive, suggesting that there may be an association between the uniformity of CD70 expression and response. Furthermore, 20 patients displayed stable diseases (Tannir et al., 2014). In all patients, substantial depletions of CD70⁺ peripheral blood lymphocytes, but not total lymphocytes, were observed after treatment with SGN-75 due to direct cytotoxicity or antibody effector functions. Due to toxicity concerns (2 patients with grade 4 idiopathic thrombocytopenic purpura), dose escalation in the weekly schedule was terminated. Three patients, treated every 3 weeks had dose limiting toxicities: Grade 3 nephrotic syndrome (1.5 mg/kg) and Grade 4 neutropenia (4.5 mg/kg) in NHL patients, and Grade 3 proteinuria (4.5 mg/kg) in an RCC patient (Tannir et al., 2014). The maximum tolerated dose was set at 3.0 mg/kg administered every three weeks and was used for enrollment of an additional 15 RCC patients for the expansion cohort, no regimen was recommended for NHL patients. Next, a phase 1b study was set up in RCC patients to evaluate the safety profile of SGN-75 (1-2 mg/kg every 21 days) in combination with the tyrosine kinase inhibitor, everolimus (10 mg daily)(NCT01677390). However, this study was discontinued and the company developed SGN-CD70A, a new CD70-blocking antibody, equipped with a different cytotoxic agent to the SGN-70 antibody backbone. SGN-CD70A is endowed with next-generation ADC technology, comprising a highly potent cytotoxic agent, called a pyrrolobenzodiazepine dimer, stably linked to a CD70-directed antibody via proprietary site-specific conjugation technology (Jeffrey et al., 2013; Sharsti Sandall et al., 2014). A phase 1 dose escalation study has recently been initiated, evaluating SGN-CD70A in CD70-positive metastatic RCC and relapsed or refractory NHL. This study is designed to evaluate the maximum tolerated dose (up to 10 mg/kg) of SGN-CD70A, administered every 3 weeks, by enrolling 95 patients.

AMG 172

AMG 172 is an IgG1 ADC of which binding and internalization into CD70-expressing tumor cells induces metaphase arrest, followed by cellular apoptosis and eventually tumor cell death (Nelson L. Jumbe, 2013). AMGEN initiated a phase 1 trial with this antibody for the treatment of relapsed or refractory clear cell RCC following at least 2 prior therapies. This study will be divided into a dose exploration part, treating patients every two or three weeks to establish a maximum tolerated dose, and a dose expansion part, enrolling an estimated total of 82 patients.

ARGX-110

ARGX-110 is another CD70-blocking IgG1 monoclonal antibody (mAb) in phase 1 whose glycoengineered Fc domain mediates targeted killing of CD70-expressing tumor cells via complement-dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCP) properties and enhanced ADCC (Silence et al., 2014). In a first study by arGEN-x, 26 patients were treated intravenously with ARGX-110 every 3 weeks with escalation doses from 0.1 to 10 mg/kg. Here, patients were only included when expression of CD70 could be detected by IHC in more than 10% of the tumor cells. In this initial dose-escalation study, ARGX-110 demonstrated a favorable safety profile with no dose-limiting toxicities seen in all the patients treated. Drug-related Grade 3 adverse events (hypoxia, anorexia, fatigue) were observed in 2 patients, whereas no immune-related toxicities could be detected. Out of the 26 patients receiving ARGX-110, 5 patients had progression free survival of more than 6 months (renal cell carcinoma, platinum-refractory ovarian cancer, head and neck cancer, myoepithelial carcinoma and mesothelioma), 1 T cell lymphoma (TCL) patient (0.1 mg/kg) achieved a complete hematological response (while skin compartment was stabilized) and 1 TCL patient (10 mg/kg) reached a biological response (Awada et al., 2014; Maerevoet, 2015). In addition ADCC and depletion of circulating CD70⁺ cells were demonstrated in all patients. Saturated target-mediated clearance was observed starting at 1 mg/kg. Furthermore, circulating Tregs were reduced by $\geq 50\%$ in most patients treated at the highest

doses. Based on these data, arGEN-X recently launched a phase 1 dose expansion study with an intermediate dose level. In contrast to the study by Seattle Genetics, solid and hematological CD70⁺ advanced malignancies are included in the study, enrolling a total of 60 patients. Additionally, a phase 1 study in patients with advanced CD70⁺ lymphomas was launched, treating them with 5 mg/kg ARGX-110 every 3 weeks. Here, a biological response was observed in 1 out of 6 patients (Maerevoet, 2015). This patient, diagnosed with angioimmunoblastic TCL, showed reduction in tumor size, reduction in lactate dehydrogenase, increased hemoglobin levels and the conversion to Coombs negativity. In addition, one other TCL patient is still enrolled in the study. Interestingly, all lymphoma patients with good response to therapy were marked with high serum sCD27 levels, prior to therapy. These results suggest a role for sCD27 as a powerful diagnostic tool for efficacy of the drug.

- **Possible Combination strategies**

Although recent therapies have shown great clinical responses as single agents, nearly always resistance develops. To circumvent this resistance, effective combination-based therapies need to be identified. Even though anti-CD70 therapy has just entered clinical trials as a monotherapy, in this section interesting combination strategies will be discussed.

Chemotherapy

Accumulating evidence suggests that tumors with a preexisting active immune microenvironment might have a better response to immunotherapy. In this regard, cytotoxic agents have shown to elicit changes in the tumor microenvironment that render cells more sensitive to an efficient immune cell attack. Although chemotherapy has long been considered immune suppressive, recent studies have shown that in addition to its direct cytotoxic effects on cancer cells, chemotherapeutic agents such as cyclophosphamide, doxorubicin, oxaliplatin, and mitoxantrone can induce immunogenic cell death and activation of anti-tumor immune responses (Chen & Emens, 2013; Ghiringhelli & Apetoh, 2014). Wang et

al. demonstrated that even after exposure of tumor cells to nonlethal doses of docetaxel, immunogenic modulation can occur, altering the tumor phenotype and also rendering cells more sensitive to CTL killing (W. Wang et al., 2015). Moreover, chemotherapy-resistant cell lines revealed enhanced CTL lysis, suggesting that the combination with immunotherapy may also improve survival rates of patients failing chemotherapy.

Recently, Agarwal et al. provided an additional rationale for combining chemotherapy with anti-CD70 therapy to overcome resistance to chemotherapy (Aggarwal et al., 2009). By using mass-spectrometry (MS) based proteomics, it was shown that expression of CD70 was associated with acquired cisplatin resistance in ovarian cancer cell lines. Most importantly, this study further demonstrated that antibodies against CD70 could inhibit the proliferation of cisplatin-resistant ovarian cancer cells *in vitro*. Also *in vivo*, the expression of CD70 was strongly associated with poor cisplatin-based chemotherapy responses in human specimens from ovarian cancer patients (Liu et al., 2013). Interestingly, an effect of chemotherapy on CD70 expression was also demonstrated in NSCLC, whereby biopsies taken before and after administration of chemotherapy revealed a strong induction of CD70 expression in the latter (Jacobs et al., 2015).

In addition, it was demonstrated that some chemotherapeutic agents feature the ability to directly activate immune effectors such as NK cells (Ghiringhelli & Apetoh, 2014). Because NK cells play a pivotal role in mediating ADCC, this can influence the efficacy of immunomodulatory antibodies. Indeed, *in vivo* studies combining Trastuzumab, a humanized mAb targeting the human epidermal growth factor receptor 2 (HER2), with Paclitaxel have already shown to improve the ADCC of Trastuzumab by rapid recruitment of NK cells (Miura et al., 2014). These studies provide a rationale for the exploration of chemotherapy in combination with anti-CD70 therapies, such as ARGX-110, equipped to induce ADCC.

Overall, these findings suggest that chemotherapy presents an option for exploration in combination with immunomodulatory antibodies. Nevertheless, further research is required to determine appropriate dosing and treatment schedule of these agents (Chen & Emens, 2013).

Radiotherapy

Radiotherapy has been proven effective in causing cancer cell death by the induction of irreversible DNA damage. In addition, accumulating evidence emphasizes the stimulatory effects of radiotherapy on the tumor-microenvironment, making it an ideal companion for combination therapies with immunotherapy. Moreover, combinations of radiotherapy with immunomodulatory antibodies such as CTLA-4 or PD-1 blocking antibodies have already shown clear synergistic potential (Yoshimoto et al., 2014). As reviewed by Shababi et al., radiotherapy can make tumors more immunogenic by enhancing antigen presentation, antigen uptake and the secretion of immunostimulatory factors (Shahabi et al., 2015). Furthermore, radiation has been reported to enhance the amount of effector T cells to the tumor site through the induction of interferons and chemokines (Soukup & Wang, 2015). In contrary to its stimulatory effects, irradiation can also induce immunosuppression by the increase in Tregs and induction of TGF- β (Soukup & Wang, 2015; Vatner et al., 2014). Combining these observations with the aforementioned role of CD70-CD27 interactions on the survival of Tregs, we speculate that a combination with anti-CD70 therapy might increase its efficacy.

Furthermore, in literature it has been described that upon irradiation, a large pool of intracellular CD70 becomes exposed onto the surface of leukemia and lymphoma cells (Hintzen et al., 1994; Lindgren et al., 2012). It is an interesting hypothesis that irradiation could increase the levels of CD70 in tumors cells. If this is the case, anti-CD70 therapy could be combined with radiotherapy to further maximize the chance for efficacy of this drug. Next to lymphoma cells, the radio-inducibility of CD70 was also demonstrated on glioma cell lines whereby an increase in cell surface expression was demonstrated in 7 out of 8 cell

lines studied (Wischhusen et al., 2002). Up to now, the underlying mechanism of this CD70 induction remains largely elusive, though it seems to be p53-independent (Wischhusen et al., 2002). Interestingly, CD70 induction upon radiotherapy appeared to play a critical role in the mediation of immune escape through the increase of apoptosis in peripheral blood mononuclear cells. The fact that CD70 expression also led to interference with immune responses, yet again suggests that this could be an ideal target for future combination strategies. One major challenge remaining is the identification of the ideal treatment schedules since various publications have demonstrated that the dose, mode of delivery and schedule of radiotherapy can cause substantially different effects on the immune response (Soukup & Wang, 2015).

Immunotherapy

For an anticancer immune response to lead to effective killing of cancer cells, a number of stepwise events must be initiated and allowed to proceed and expand iteratively, as nicely illustrated in the cancer-immunity cycle (Chen & Emens, 2013). In this cycle, neo-antigens must be captured and presented, resulting in the priming and activation of effector T cell responses against these tumor-associated antigens. Targeting multiple steps in this cancer-immunity cycle might therefore maximize the efficacy of the drug. One such example is the combination of anti-CTLA-4 with PD-1 targeted inhibition which has already demonstrated durable partial and complete responses in melanoma (Callahan et al., 2014). Combining CD70-CD27 blockade with other immune checkpoint inhibitors might therefore be another way to reinvigorate and expand preexisting anticancer immune responses. Unfortunately, abolishing the suppressive factors in the tumor microenvironment is only one step in this cancer-immunity cycle and still requires elimination of cancer by activated T cells. Therefore, another interesting approach could be to not only overcome immunosuppression, but also to combine this with enhancers of T cell activation to achieve maximum killing efficacy. Hence, it would be reasonable to speculate that vaccines, such as peptide-based or dendritic cell-based vaccines, could also serve as an ideal partner for

anti-CD70 therapy (Deschoolmeester et al., 2013). Interestingly, through this augmentation of immune responses, an enhancement of ADCC might also be expected. Finally, certain cytokines such as IL-2, IL-15 and granulocyte macrophage colony stimulating factor are also known to enhance the NK cell or neutrophil activity, and as such, have the potential to enhance the activity of CD70-blocking antibodies (Lopez et al., 1986; Van den Bergh et al., 2014). The main hurdle for these sets of combination are the immune-related toxicities, which necessitates preclinical studies aiming to minimize these side effects.

5. Discussion: are we targeting the right molecule?

Biological and clinical evidence as described above support further development of CD70 blocking antibodies for anti-tumor immunity. Nevertheless, expression of CD70 on tumor cells or antigen-presenting cells has also been described to have anti-tumoral effects (Arens et al., 2004; Bak et al., 2012; Couderc et al., 1998; Glouchkova et al., 2009; Kelly et al., 2002; Lorenz et al., 1999). This raises the question if CD70 blocking antibodies might also have the opposite effect on anti-tumoral immunity by blocking the stimulatory function of CD70 on antigen-presenting cells and NK cells or by depleting activated T cells, as suggested by the CD27-agonistic antibody, CDX-1127 (Thomas et al., 2014). Although depletion of CD70⁺ peripheral blood lymphocyte could be observed after SGN-75 therapy, no difference in the total amount of lymphocytes was seen. In addition, no adverse reactions due to eradication of CD70⁺ immune cell populations by the blocking antibodies are known to date. A possible explanation for this might be the transient state of CD70 expression on immune cell populations. In addition, the prominent immunostimulatory activity of the CD70/CD27 axis in *de novo* induced immune responses seems paradoxically opposite from its role in the established tumor microenvironment whereby CD27 triggering can lead to tumor progression through recruitment of CD27⁺ Tregs, apoptosis of lymphocytes and T-cell exhaustion. This might possibly be explained by the overexpression of CD70 on these tumor cells since accessibility of CD70 appears to be a critical component for the effects on T cell function.

Furthermore, it should be noted that the observed stimulatory effects of CD27 are mostly described in experiments using murine models. For example, a study by Kelly et al. showed NK-cell mediated tumor rejection upon CD70 expression by tumor cells (Kelly et al., 2002). This was mostly established by the interaction of CD70 with CD27-expressing NK cells, providing a key link between innate and adaptive immunity. Of interest here is that CD27 is a constitutive marker of murine NK cells, whereas in human peripheral blood, only 30-40% of NK cells express CD27. Moreover, human circulating CD27⁺ NK cells seem to exert low cytolytic potential where CD27⁻ NK cells contain high levels of perforin and granzyme B. In addition, some discrepancies might come from the setting used to study tumor immunology in murine models. When established by subcutaneous or intravenous injection of cancer cell lines, the CD70/CD27 axis seems to result in increased effector CD4⁺ and CD8⁺ T cell function (Aulwurm et al., 2006; Lorenz et al., 1999; Roberts et al., 2010). Conversely, an opposite outcome presented itself when solid tumor fragments were transplanted into mice with the promotion of tumor growth by CD70-CD27 triggering, which was primarily established by the increased frequency of intratumoral Tregs (Claus et al., 2012)(Reviewed in (Riether et al., 2012)). Although to date there is no evidence that CD70 blocking antibodies harm the patient's immune system in general and more specific the anti-tumor response, it is important to keep monitoring these potential adverse effects.

6. Conclusion

Over the last years, new insights into the CD70/CD27 axis have been gained, unraveling its unique properties in the tumor-microenvironment. Through the constitutive expression of CD70 on tumor cells, persistent signaling of CD27 in the tumor-microenvironment has been shown, mediating immune escape by T cell apoptosis, T cell exhaustion and Treg survival. Additionally, it is becoming clear that blocking this pathway can also abolish tumor proliferation, mediated through the co-expression of CD70 and CD27 on malignant cells of hematological lineage. The immune inhibitory effects, the constitutive expression of

CD70 on tumor cells and the restricted expression in normal lymphoid tissue can be exploited to direct ADCC-inducing antibodies. Hence, blocking this pathway is currently a hot topic in clinical trials and holds great potential as monotherapy. In addition, anti-CD70 therapy offers many opportunities for rational combination strategies with conventional therapy as well as immunotherapy.

Acknowledgements

This work was supported by the TGO fund, granted by the agency for Innovation by Science and Technology (IWT). We would also like to express our gratitude to the tumor biobank at the Antwerp University Hospital for the close collaboration.

Conflict of interest statement

Only one author reports a potential conflict of interest: Karen Silence, Biomedical scientist PhD and project manager at arGEN-X.

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Tables

Table 1: Overview of CD70 expression by immunohistochemistry on hematological and solid malignancies.

Malignancy	Reference	IHC method	CD70 Antibody	CD70 expression		Threshold for
				CD70 ⁺ /total	Percentage CD70 ⁺	CD70 positivity
Hematological malignancies						
Non-Hodgkin lymphoma						
	(McEarchern et al., 2008)	IHC-FFPE	mAb SG-21.1 C1	71/119	60%	n.s.
	(Tannir et al., 2014)	IHC-FFPE	n.s.	82/107	77%	n.s.
Diffuse large B cell lymphoma	(Lens et al., 1999)	IHC-Fr	mAb 2F2	15/21	71%	20%
	(Adam et al., 2006)	IHC-Fr	mAb HNE5.1	4/6	67%	n.s.
Follicular lymphoma	(Lens et al., 1999)	IHC-Fr	mAb 2F2	6/18	33%	20%
	(Adam et al., 2006)	IHC-Fr	mAb HNE5.1	0/8	0%	n.s.
Mantle cell lymphoma	(Lens et al., 1999)	IHC-Fr	mAb 2F2	1/4	25%	20%
Burkitt lymphoma	(Lens et al., 1999)	IHC-Fr	mAb 2F2	1/4	25%	20%
CAEBV associated T cell lymphoma	(Shaffer et al., 2012)	IHC-FFPE	n.s.	1/1	100%	n.s.
Hodgkin lymphoma						
	(Gruss & Kadin, 1996)	IHC-?	n.s.	n.s.	96%	n.s.
	(McEarchern et al., 2008)	IHC-FFPE	mAb SG-21.1 C1	23/24	97%	n.s.
Leukemia						
B-cell chronic lymphocytic leukemia	(Lens et al., 1999)	IHC-Fr	mAb 2F2	3/6	50%	20%
	(Adam et al., 2006)	IHC-Fr	mAb HNE5.1	0/2	0%	n.s.
Multiple myeloma						
	(McEarchern et al., 2008)	IHC-FFPE	mAb SG-21.1 C1	9/22	41%	n.s.
Solid malignancies						
Renal cell carcinoma						
	(McEarchern et al., 2008)	IHC-FFPE	mAb SG-21.1 C1	14/20	70%	n.s.
	(Tannir et al., 2014)	IHC-FFPE	n.s.	111/127	87%	n.s.
Clear cell	(Junker et al., 2005)	IHC-Fr	mAb HNE5.1	41/41	100%	n.s.
	(Diegmann et al., 2005)	IHC-Fr	mAb HNE5.1	10/10	100%	n.s.
	(Adam et al., 2006)	IHC-Fr	mAb HNE5.1	16/20	80%	n.s.
	(Law et al., 2006)	IHC-Fr	mAb 2F2	71/131	54%	25%
	(Ryan et al., 2010)	IHC-FFPE	1C1/5D12Ab	189/230	81%	1%.
	(Jilaveanu et al., 2012)	IHC-ISH	n.s.	113/232	49%	v>20.1
Papillary	(Junker et al., 2005)	IHC-Fr	mAb HNE5.1	1/9	5%	5%
	(Adam et al., 2006)	IHC-Fr	mAb HNE5.1	0/3	0%	n.s.
	(Law et al., 2006)	IHC-Fr	mAb 2F2	9/23	39%	25%
	(Ryan et al., 2010)	IHC-FFPE	1C1/5D12Ab	4/8	50%	1%
	(Jilaveanu et al., 2012)	IHC-ISH	n.s.	9/46	19%	v>20.1
Chromophobe	(Junker et al., 2005)	IHC-Fr	mAb HNE5.1	1/5	20%	n.s.
	(Law et al., 2006)	IHC-Fr	mAb 2F2	0/6	0%	25%
Oncocytomas	(Junker et al., 2005)	IHC-Fr	mAb HNE5.1	0/3	0%	n.s.
	(Adam et al., 2006)	IHC-Fr	mAb HNE5.1	0/1	0%	n.s.
	(Jilaveanu et al., 2012)	IHC-ISH	n.s.	1/20	5%	v>20.1
Sarcomatoid	(Adam et al., 2006)	IHC-Fr	mAb HNE5.1	0/1	0%	n.s.
	(Jilaveanu et al., 2012)	IHC-ISH	n.s.	6/11	10%	v>20.1
Brain carcinoma						
	(Adam et al., 2006)	IHC-Fr	mAb HNE5.1	0/1	0%	n.s.
	(Ryan et al., 2010)	IHC-FFPE	1C1/5D12Ab	6/59	10%	1%
Glioblastoma	(Wischhusen et al., 2002)	IHC-Fr	n.s.	5/12	42%	n.s.
Astrocytoma	(Wischhusen et al., 2002)	IHC-Fr	n.s.	3/4	75%	n.s.

Nasopharyngeal carcinoma						
	(Ryan et al., 2010)	IHC-FFPE	1C1/5D12Ab	18/82	22%	1%
EBV-associated NPC	(Agathangelou et al., 1995)	IHC-Fr	Ki24	16/18	89%	n.s.
Mesothelioma						
	(Hishima et al., 2000)	IHC-Fr	mAb HNE5.1	0/2	0%	1%
Hepatocellular carcinoma						
	(Adam et al., 2006)	IHC-Fr	mAb HNE5.1	0/1	0%	n.s.
Esophagus carcinoma						
	(Hishima et al., 2000)	IHC-Fr	mAb HNE5.1	0/5	0%	1%.
Pancreatic carcinoma						
Esophagus carcinoma	(Ryan et al., 2010)	IHC-FFPE	1C1/5D12Ab	35/140	25%	1%
Colon carcinoma						
	(Adam et al., 2006)	IHC-Fr	mAb HNE5.1	0/2	0%	n.s.
	(Ryan et al., 2010)	IHC-FFPE	1C1/5D12Ab	17/194	9%	1%
Breast carcinoma						
	(Adam et al., 2006)	IHC-Fr	mAb HNE5.1	0/11	0%	n.s.
	(Ryan et al., 2010)	IHC-FFPE	1C1/5D12Ab	5/204	2%	1%
Ovarian carcinoma						
	(Aggarwal et al., 2009)	IHC-FFPE	LP-28809	10/10	100%	0.1%
	(Ryan et al., 2010)	IHC-FFPE	1C1/5D12Ab	37/241	15%	1%
Thyroid carcinoma						
	(Adam et al., 2006)	IHC-Fr	mAb HNE5.1	0/1	0%	n.s.
Lung carcinoma						
	(Hishima et al., 2000)	IHC-Fr	mAb HNE5.1	0/17	0%	1%
	(Adam et al., 2006)	IHC-Fr	mAb HNE5.1	1/19	5%	n.s.
Adenocarcinoma	(Jacobs et al., 2015)	IHC-FFPE	4B12	3/32	9%	10%
	(Ryan et al., 2010)	IHC-FFPE	1C1/5D12Ab	17/172	10%	1%
Squamous carcinoma	(Jacobs et al., 2015)	IHC-FFPE	4B12	4/15	27%	10%
Neuro-endocrine carcinoma	(Jacobs et al., 2015)	IHC-FFPE	4B12	1/1	100%	10%
Thymic carcinoma						
	(Hishima et al., 2000)	IHC-Fr	mAb HNE5.1	8/27	30%	1%
Melanoma						
	(Adam et al., 2006)	IHC-Fr	mAb HNE5.1	0/1	0%	n.s.
	(Ryan et al., 2010)	IHC-FFPE	1C1/5D12Ab	15/96	16%	1%

Abbreviations: IHC, immunohistochemistry; FFPE, formalin-fixed paraffin embedded; Fr, frozen; ISH, *in situ* hybridization; mAb, monoclonal antibody; n.s., not specified; EBV, Epstein-Barr virus; CAEBV, chronic active EBV-infection; NPC, nasopharyngeal carcinoma; v, value.

Table 2: Characteristics of ongoing CD70-targeting clinical trials.

	SGN-CD70A	AMG 172	ARGX-110
Trial identifier	NCT02216890	NCT01497821	NCT01813539
Mechanism of action	CD70 blocking Toxin-mediated	CD70 blocking Toxin mediated	CD70 blocking ADCP, CDC, enhanced ADCC
Phase	1	1	1
Dose and Schedule	Dose escalation up to 10 mg/kg Every 3 weeks	Dose escalation Every 2-3 weeks	5 mg/kg Every 3 weeks
Inclusion criteria	CD70+ malignancies (n=75) restricted to metastatic RCC and relapsed/refractory NHL	Relapsed/refractory clear cell RCC; ≥2 prior treatments (n=82)	CD70+ Advanced malignancies (n=60) (hematological and solid malignancies)
Internalization dependent	Yes	Yes	No

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; CDC, complement-dependent cytotoxicity; RCC, renal cell carcinoma; NHL, non-Hodgkin lymphoma.

8. Legends of Figures

Figure 1: CD70-CD27 pathway. Diagram showing the CD27-NF κ B pathway, mediated by TRAF2, and the CD27-c-Jun kinase pathway, mediated by TRAF5, resulting in survival, proliferation and differentiation signals. On the left, cytoplasmic binding of CD27 to Siva is illustrated, leading to caspase-mediated apoptosis. NIK, NF κ B inducing kinase; IKK, I κ B kinase; JNK, c-jun N-terminal kinase; TRAF, TNF receptor-associated factor 2.

Figure 2: CD70-CD27 signaling in the tumor-microenvironment. Four different mechanisms of immune escape, mediated through the constitutive expression of CD70 on the tumor cells, are illustrated. A: proliferation of naturally occurring Tregs; B: induction of FOXP3 expression in CD4⁺CD25⁻ Tregs; C: caspase-mediated apoptosis of T cells through the cytoplasmic binding of Siva; D: induction of T cell exhaustion. nTreg, natural regulatory T cell; iTreg, induced regulatory T cell.