NKG2A: A Novel Immune Checkpoint Protein for Cancer Treatment

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Abstract: Immune checkpoint inhibitor (ICI) drugs have been figured prominently in various of cancer immunotherapy. Immune checkpoint inhibitor monoclonal antibodies (mAbs) targeting cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1) or its ligand (PD-L1), have achieved unprecedented improvements. Nonetheless, due to some issues such as tumor resistance, genetic heterogeneity, and its intricacy of immune regulatory pathways, immunotherapy remains a major challenge. As a result, it's critical to discover further about immunological checkpoints and the application of their inhibitors in clinical practice. The potential inhibitory CD94/NKG2A receptor has been explored in recently, which involve in the stimulation of both natural killer (NK) and CD8+ T cell immunological activity, as well as predominantly link to immune cell-tumor interaction. However, the specific mechanisms for immune regulation, NKG2A-targetd inhibitors and related clinical trials are still underestimated. Therefore, we'll look at the basic structure and function of CD94/NKG2A, and its immune regulatory mechanisms, as well as the current NKG2A-targeted clinical results in the review.

1 INTRODUCTION

Immune checkpoint protein originally acts as mediators for protection of normal tissues from carcinogenesis. However, it is found that tumor cells utilize some of the immune checkpoints as a significant channel for evasion of immune surveillance and immune resistance. To date, immune checkpoint inhibitor (ICI) drugs that efficiently block or modulate the ligand-receptor interaction have been developed to improve the treatment for cancers. The ICI targeting PD-1/PD-L1 and CTLA-4 were showed therapeutically potent and improved the clinical benefits for patients. Seven of them therefore were approved by the US Food and Drug Administration (FDA), involving one for CTLA-4 (ipilimumab), three for PD-1 (nivolumab, pembrolizumab, cemiplimab) and another three for PD-L1 (atezolizumab, avelumab, duralumab) (Verma, Sprave, Haque, et al. 2018). Although ICIs displayed remarkable outcomes in clinical trials for cancers, e,g non-small cell lung carcinoma (NSCLC), melanoma, and relapsed or refractory Hodgkin's lymphoma, it can only benefit a small number of patients due to the complicate mechanisms of different types of tumor micro-environment (TME) for protection of tumor against

immune responses (Darvin, Toor, Sasidharan Nair, Elkord, 2018). What's worse, some of the patients who are treated with immune checkpoints inhibitors risk serious immune-related adverse events (irAEs), and hyper-progression (Feng, Roy, Masson, Chen, Humphrey, Weber, 2013). Therefore, more effective and safe molecular targets and drugs are essential for improvement of ICI discovery and treatments. NKG2A, an inhibitory receptor that regulates both innate and adaptive immunity through subsets of T cells and NK cells, that injects new blood into cancer immunotherapy. NKG2A often expresses on the membranes of NK cells and T cell subsets as a heterodimeric proteins by linking with invariant CD94 polypeptide disulfide (Boyington, Riaz, Patamawenu, Coligan, Brooks, Sun, 1999). NK cells and T cells have significant effect on limiting both tumor progression and metastasis (Zaghi, Calvi, Marcenaro, Mavilio, Di Vito, 2019). The recognition of human histocompatibility leukocyte Ag-E (HLA-E) on tumor cells by CD94/NKG2A complex inhibits certain functions of NK cells and particular types of T cells, thus speeding up tumor escape from the immune system5.In this case, tumor immunity can be greatly enhanced by the inhibition of CD94/NKG2A or the ligands, although there are limited approved medicines, established researches and trials about this

receptor. Here, we review the foundational structure of NKG2A, involving it genetic information, protein domain and regulatory mechanisms, as well as its advances and updates in pan-cancers.

2 NKG2A/CD94

2.1 The Structure of NKG2A

NKG2A is an inhibitory member of the NKG2 family of receptors6, consisting of 233 amino acid. NKG2 is a C-type lectin-like receptor family including seven subunit proteins, NKG2A, B, C, D, E, F, and H (Sullivan, Clements, Beddoe, et al, 2007). Previous results have demonstrated that NKG2A shows great similarity in genomic organization and sequence with NKG2-C, -E and -F, having the same transcriptional orientation as theirs (Borrego, Kabat, Kim, et al. 2002). However, NKG2A transcript involves an unique extra 5 untranslated exon (Plougastel, Trowsdale, 1998). Its C-type lectin domain is possessed by a type II integral membrane protein (Glienke, Sobanov, Brostjan, et al, 1998). The NKG2A molecule carries two immunoreceptor tyrosine-based inhibitory motifs (ITIMS) in the intracytoplasmic tail nearly identical to those of the inhibitory KIRs (Borrego, Kabat, Kim, et al. 2002) and forms lengthened disulfide-linked heterodimers with the invariant CD94 protein (Walter, Petersen, 2017). Recent researches have also shown that NKG2A is expressed in combination with other proteins in some NK cells (Figure 1A) and T cell subsets (Figure 1B). The NKG2A receptor has been found on nearly half of the peripheral NK cells which are predominantly presented in the CD56high fraction that contains the more immature cells (Borst L, Burg SH van der, Hall T van. 2020). Moreover, intra-tumoral NK cells express rather high frequencies of NKG2A (van Montfoort, Borst, Korrer, et al, 2018). The expression of NKG2A In CD8 T cells has been strictly regulated since peripheral cells hardly display this receptor, while the majority of intra-tumoral T cells, particularly those in the tumor microenvironment, exhibit NKG2A (van Montfoort, Borst, Korrer, et al, 2018, Hamid, Wang, Yao, et al, 2019).
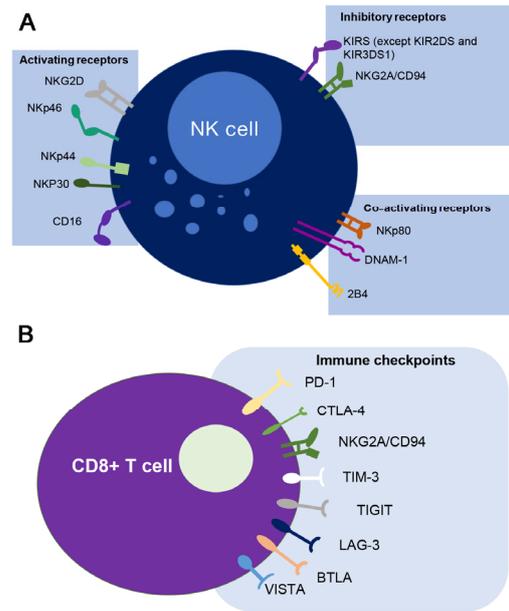

Figure 1. The expression and interaction of NKG2A in NK cell (A) and Tcell (B).

The NKG2A ectodomain, with the overall dimensions 54*33*29 A, is consist of two perpendicular beta sheets formed by 8 Beta strands, plus 2 alpha helices (residues 140–149 and 160–169) on the opposite face of the promotor (Sullivan, Clements, Beddoe, et al, 2007). The three bonds stabilizing the NKG2A fold are Cys119-Cys130 for the connection between the beta2 strand and the N-terminal loop; Cys147-Cys229 for the connection between beta7 strand and alpha1 helix; and Cys208-Cys221 for the connection between beta5 strand to loop 6 (Sullivan, Clements, Beddoe, et al, 2007). Therefore, NKG2A is in the same structural homologous series with CD94 (Sullivan, Clements, Beddoe, et al, 2007).

The CD94 molecule, a subunit of CD94/NKG2A receptor with general dimension of 42*37*33 A in approximation, is made up of an antiparallel beta sheet with strands 1, 2, 7, an antiparallel beta sheet with strands 4, 3, 5, and 6, and an alpha helix after strand 2 (Boyington, Riaz, Patamawenu, Coligan, Brooks, Sun, 1999). It has four intrachain disulfide linkages, including three invariant disulfides (Cys61–Cys72, Cys89–Cys174, and Cys152–Cys166) which presented in long-form C-type lectin (Day, 1994). Cys59-Cys70 which is the fourth disulfide, links with N-terminal beta strands and is found uniquely in the molecular structure of CD94 (Boyington, Riaz, Patamawenu, Coligan, Brooks, Sun, 1999). Cys58, the lone interstitial cysteine not found in interchain disulfide pairing, is supposed to combine with the

Cys116 in NKG2A, resulting in the interchain disulfide in the CD94/NKG2A heterodimer (Boyington, Riaz, Patamawenu, Coligan, Brooks, Sun, 1999).

The CD94/NKG2A dimer has the dimension 75*42*38 A with a large dimer interface containing roughly 1500 A2 of buried surface area (BSA) (Sullivan, Clements, Beddoe, et al, 2007), at which CD94 and NKG2A contribute 69% and 31% respectively (Petrie, Clements, Lin, et al, 2008). The interaction between the alpha2 helix of NKG2A and the complementary prolonged loop region of CD94 greatly leads to the asymmetry at the CD94/NKG2A interface (Sullivan, Clements, Beddoe, et al, 2007). Additionally, there are two of the amino acids in CD94/NKG2A locating on the presumed HLA-E binding site, position 197 in loop 5 (Glu) and position 225 within loop 7 (Ile) (Sullivan, Clements, Beddoe, et al, 2007).

According to recent researches, tumor infiltration NK cells and CD8+ T cells express an abnormal amount of NKG2A which contributes to the poor cancer prognosis (Zaghi, Calvi, Marcenaro, Mavilio, Di Vito, 2019). By analyzing the tissue-infiltrating leukocyte (ITL) from normal livers, intratumor tissues (IT), peritumor tissues (PT) and intratumor tissues (IT), Cheng Sun et al had found that the expression of NKG2A in NK cells from IT was dramatically increased in comparison with those cells in healthy livers and PT which also relates to NK cell exhaustion and to great extent, results in a shorter overall survival (OS) of patients with hepatocellular carcinoma (Sun, Xu, Huang, et al, 2017). Furthermore, the upregulation on NK cells by NKG2A in patients with lung cancers can act as a biomarker of tumor metastatization (NK Cell Phenotypic Modulation in Lung Cancer Environment, 2021). Besides, the direct interaction between NK cells and intratumor stromal cells gives rise to the pathogenic and phenotypic mutation of NK cell in lung cancer as well as invasive breast cancer where an increment in the expression of NKG2A and a lessened expression of the NKRs NKp30, NKG2D, DNAM-1, and CD16 have been observed (Galland, Vuille, Martin, et al, 2017, Mamessier, Sylvain, Thibult, et al, 2011).

2.2 The Ligand of NKG2A in Both NK and T Cells

The primary ligand for CD94/NKG2A inhibitory receptor is the human major histocompatibility complex class Ib (MHC-Ib) molecule, HLA-E (Braud, Allan, O'Callaghan, et al, Borrego, Ulbrecht, Weiss, Coligan, Brooks, 1998,) and its mouse ortholog Qa-1b

(Borst L, Burg SH van der, Hall T van. 2020). This class Ib protein specifically binds and presents an immensely associated set of nonameric peptides generated from the signal sequences of class I molecules (Braud, Allan, O'Callaghan, et al, 1998, Braud, Yvonne Jones, McMichael. 1997, Lee, Llano, Carretero, et al, 1998), which is different from class Ia molecules that exhibit a broad range of peptide ligands. In addition, it contains only two functional alleles present in humans (the HLA-E*01:01 and the HLA-E*01:03 variants) (Borst L, Burg SH van der, Hall T van. 2020). These 2 alleles can be distinguished from each other only by an individual amino acid at position 107 which is arginine (01:01) or glycine (01:03) (Borst L, Burg SH van der, Hall T van. 2020). The expression of HLA-E is overall common whereas relatively low in normal tissues with exceptions of high level of expression in trophoblast cells in the placenta and ductal epithelial cells in the testis and epididymis due to the effect of HLA-E in immune tolerance (Wei, Orr, 1990, van Hall, André, Horowitz, et al, 2019). In contrast, the amount of HLA-E exert on tumor cells are abnormally increased in lung, kidney, pancreas, stomach, colon, head and neck, liver, melanoma, prostate, and rectal tumor tissues (van Montfoort, Borst, Korrer, et al, 2018, Gooden, Lampen, Jordanova, et al, 2011). It has been reported that high HLA-E expression can be associated with a poor prognosis in colorectal carcinoma, breast and ovarian carcinoma (Gooden, Lampen, Jordanova, et al, 2011, Levy, Bianchini, Von Euw, et al, 2008). Nevertheless, a favorable connection between expression of HLA-E and survival time has been recognized in patients with glioblastoma (Kren, Slaby, Muckova, et al, 2011).

Joseph D.Miller et al have indicated that the position 5 Arg side chain in HLA-E performs as a dominant contact for interaction with CD94/NKG2A receptor, which acts as one of the main contact residues together with P8 amino acids for this interaction (Miller, Weber, Ibegbu, Pohl, Altman, Jensen, 2003). For mechanism, the tyrosine phosphorylation of ITIMs and following recruitment and activation of phosphatases (SHP-1 and SHP-2) then characterizes the ligation of inhibitory receptors, thus causing the inhibition of various NK cell-mediated effector functions (Burshtyn, Scharenberg, Wagtmann, et al, 1996). The length of amino acid between the two ITIMs in NKG2A is about 25 peptides, which is regarded to be appropriate for the occupation of tandem SH2 with phosphatases6. Also, for the maximum phosphatase catalytic activity, it is necessary to activate SH2 domains of SHP-1/-2 simultaneously (Pluskey, Wandless, Walsh,

Shoelson, 1995). Previous discovery had shown that HLA-E complexed to the peptide correlating to the leader sequence peptide derived from HLA-G-bound CD94-NKG2A has the affinity of 0.94 mM (Kaiser, Barahmand-pour, Paulsene, Medley, Geraghty, Strong, 2005) and the peptide structure was found to affect binding affinity (Miller, Weber, Ibegbu, Pohl, Altman, Jensen, 2003).

2.3 Current Statue of NKG2A in Basic Research

Since CD94/NKG2A receptor is expressed by not only NK cells but also a subsets of T cells such as activated α β CD8pos T-cells, $\gamma\delta$ T cells, and NK-T cells34, the blockade of CD94/NKG2A can effectively unleash the reactivity of immune cells involving several types of cytotoxic lymphocytes (Figure2)., resulting in triggering their antitumor potentials and strengthening tumor control (Zaghi, Calvi, Marcenaro, Mavilio, Di Vito, 2019).
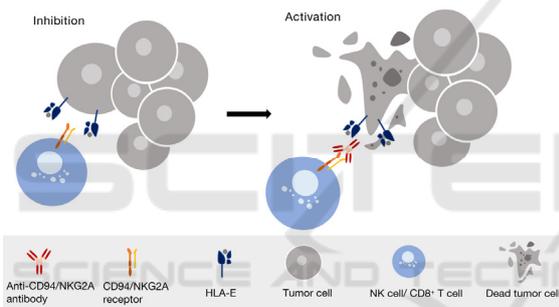

Figure 2. The mechanism of NKG2A inhibitor for cancer treatment

Monalizumab/IPH2201 is a humanized and clinical stage anti-NKG2A monoclonal antibody(mAb) which was initially developed in mice

(Zaghi, Calvi, Marcenaro, Mavilio, Di Vito, 2019). It have revealed therapeutic effect on immunodeficient mice with human leukemia (Effects of anti-NKG2A antibody administration on leukemia and normal hematopoietic cells, 2021), leading to its possibility of its development in phase I-III clinical trials targeting solid tumors and hematologic. According to the Tg32 mouse PK assay, the monalizumab has a binding affinity of 48.1 ± 3.1nM, NK cell killing efficacy of 1.5 ± 0.78 μg/ml and an approximately 17-day plasma PK half-life in Tg32 mouse. It is recently investigated for its efficacy and toxicity in the treatment of different forms of cancers, such as gynecological and squamous cell carcinoma of the head and neck (SCCHN) (Spinosa, Musial-Siwek, Presler, et al, 2021). Since CD94/NKG2A receptors usually co-express with PD-1 (André, Denis, Soulas, et al, 2018), monalizumab are recently examined with anti-PD(L)1 antibodies (durvalumab and nivolumab) (Spinosa, Musial-Siwek, Presler, et al, 2021), which have also been registered in NIH gov.clinical trial (https://clinicaltrials. gov/ct2/home)

3 CLINICAL APPLICATION OF NKG2A INHIBITOR, MONALIZUMAB

Nowadays, the safety and efficacy of monalizumab is investigated and tested. A number of clinical trials for the treatment of different types of cancer have already completed and shown effective results, including Gynecologic cancer (NCT02459301), colorectal cancer (NCT02671435), recurrent or metastatic head and neck cancer (NCT02643550) and chronic lymphocytic leukemia (NCT02643550), also listed in table1 (Table 1).

Table 1: Completed clinical trials related to monalizumab (IPH2201).

Target	Drug	Pivotal Indication	Trials No.	Phase	Most recent result
NKG2A	Monalizumab (IPH2201)	Gynecologic cancer	NCT024 59301	2	Monolizumab (10 mg/kg i.v. every two weeks) is well tolerated in individuals who have already been treated for with gynecologic malignancies. There are mild related adverse events and no dose-limiting cytotoxicity. Short-term disease stabilization is observed.
PD-L1 NKG2A VEGF EGFR	Durvalumab Monalizumab mFOLFOX6 Bevacizumab Cetuximab	metastatic microsatellite-stable colorectal cancer (MSS-CRC)	NCT026 71435	1 and 2	In advanced/metastatic MSS-CRC, the combined therapy had a manageable safety profile, with no dose-limiting toxicity and DMCB showed promising preliminary activity. Most of the patients treated give partial response or have a chronic disease.

NKG2A EGFR	Monalizumab Cetuximab	recurrent or metastatic head and neck cancer	NCT026 43550	1 and 2	the combination of monalizumab and cetuximab is safe for the patients with recurrent or metastatic head and neck cancer. Most of the patients respond partially or have a stable disease.
NKG2A	monalizumab Ibrutinib	Chronic Lymphocytic Leukemia	NCT025 57516	1/2	The combination of monalizumab and ibrutinib shows dose-limiting toxicity. Most patients show partial response or have a stable disease. The most common adverse events is Diarrhea. Due to its termination, the results have not been completed.

The purpose of NCT02459301 is to explore and determine the recommended phase II dose (RP2D) of monalizumab which is analyzed as a separate agent for treatment for patients with advanced, recurrent, or metastatic gynecologic malignancies and its clinical activity, pharmacokinetics, pharmacodynamics, safety, and immunogenicity (Tinker, Hirte, Provencher, et al, 2019). The data from this trial indicates the recommended dose to be 10 mg/kg every 2 weeks (Tinker, Hirte, Provencher, et al, 2019). Moreover, although monalizumab solely did not induce treatment responses, it shows promising activities: short-term stabilization, minimal drug toxicities and high treatment efficacy (Tinker, Hirte, Provencher, et al, 2019). The clinical trials of NCT02671435 combining monalizumab with durvalumab (MEDI4736, anti-PD-L1 mAb) which also investigates solid tumors demonstrates favorable results again. The toxicity and tolerability of monalizumab as a single agent and in combination with durvalumab had been tested in a non-responder cancer to single-agent anti-PD-1/PD-L1 therapy called metastatic microsatellite-stable colorectal cancer (MSS-CRC), (Wainberg, Diamond, Curigliano, et al, 2020). The updated data confirms the excellent tolerance of this therapy without dose-limiting toxicity and the majority of participants display partial responses or have stable diseases (Wainberg, Diamond, Curigliano, et al, 2020). Another clinical trial related to solid tumor is the trial NCT02643550, a multicenter single arm study to evaluate the combination of monalizumab and cetuximab(anti-EGFR) in patients with recurrent and/or metastatic head and neck cancer (R/M SCCHN) (Cohen, Bauman, Salas, et al, 2020). The preliminary data revealed the acceptable safety of this therapy and early, deep and durable responses in participants (Cohen, Bauman, Salas, et al, 2020). The therapy showed encouragement of progress free survival (PFS) and overall survival (OS) in both 10 naïve and 10 pretreated patients, as well as higher activity in platinum-resistant, HPV positive and negative patients than cetuximab alone based on historical data (Cohen, Bauman, Salas, et al, 2020). Importantly, these information warrant the increased attempt of combination of monalizumab and cetuximab in the treatment for SCCHN.

Different from these clinical trials studying solid tumors, phase I/II clinical trial (NCT02557516) which analyses the combination of monalizumab with irutinib, a Bruton's tyrosine kinase inhibitor already used in the treatment of the Chronic Lymphocytic Leukemia (CLL) which is a hematologic malignancy, aims at seeking for a long-run therapeutic benefit for patients suffering from CLL (Innate Pharma, 2021). The trial was designed to justify the assumption that the coeffect of ibrutinib and monalizumab will give rise to complete response (CR) rate, particularly CR without minimal residual disease (MRD), since this has been proved to be correlated with permanent clinical benefit (Innate Pharma, 2021). Unfortunately, it had been terminated due to different factors including adverse events, disease progression, physician decision and sponsor decision (Innate Pharma, 2021). But it still left valuable data: most of the treated patients have a reverse event of diarrhea and show partial responses or have a stable disease. Furthermore, the result revealed dose-limiting toxicity (Innate Pharma, 2021).

Apart from the clinical trials above, there are several ongoing trials for the investigation of monalizumab which are still enrolling patients (Table 2). Different combinations with anti-EGFR, anti-PD-L1, morpholino-pyrimidine-based inhibitor and chemotherapy are tested in different cancer indications, including patients with unresectable stage III NSCLC (NCT03822351) and patients with PD-1 therapy-resistant NSCLC (NCT03833440), as well as resectable early-stage (II-IIIA) NSCLC, or patients with advanced squamous cell carcinoma of the head and neck (NCT04590963, NCT03088059), and also a trial tested monalizumab alone in patients with Advanced or Metastatic Hematological or Solid Malignancies (NCT04333914).

Table 2: The ongoing clinical trials related to monalizumab (IPH2201).

Target	Drug	Indication	Clinical trial no.	Phase	Recruitment status	First posted date	Last update posted	Estimated Study completion date
NKG2A EGFR	Monalizumab Cetuximab	Squamous Cell Carcinoma of the Head and Neck	NCT04590963	3	Recruiting	Oct.19th 2020	Oct.29th 2021	Mar.28th 2024
PD-L1 CD73 NKG2A	Durvalumab Oleclumab Monalizumab	Stage III Non-Small Cell Lung Cancer Unresectable	NCT03822351	2	Active, not recruiting	Jan.30th 2019	Aug.4th 2021	Jul.11th 2023
NKG2A	Monalizumab	Advanced or Metastatic Hematological or Solid Tumor	NCT04333914	2	Active, not recruiting	Apr.3rd 2020	Aug.5th 2021	Dec.2021
PD-L1 CD73 NKG2A	Durvalumab Oleclumab Monalizumab	Non-small Cell Lung Cancer	NCT05061550	2	Not yet recruiting	Sept.29th 2021	Sept.29th 2021	Feb.19th 2025
PD-L1 NKG2A CD73	Durvalumab Monalizumab Oleclumab Ceralasertib Docetaxel	Non-small Cell Lung Cancer	NCT03833440	2	Recruiting	Feb.7th 2019	Apr.1st 2021	Feb.2024
NKG2A EGFR PD-L1 PD-1	Monalizumab Cetuximab Anti-PD(L)1	Head and Neck Neoplasms	NCT02643550	1,2	Active, not recruiting	Dec.31st 2015	Feb.9th 2021	Sept,2022
NKG2A PD-L1	Monalizumab Duvalumab	Carcinoma, Squamous Cell of Head and Neck	NCT03088059	2	Recruiting	Mar.23rd 2017	Nov.6th 2019	Dec.2021

4 CONCLUSIONS

The main challenges of immunotherapy are to raise the population of responding patients, as well as overcoming tumor resistance (Borst L, Burg SH van der, Hall T van. 2020). The NK cell and T cell immunotherapy are fast-growing field with remarkable contribution to cancer treatment. Here, CD94/NKG2A, as a novel immune checkpoint protein expressed on subsets of NK and T cells, has its upregulation related to upregulation of its ligand, HLA-E, and immune cell exhaustion. The abnormal expression of both CD94/NKG2A and its ligand in intratumor region is negatively correlated to cancer prognosis, OS and DFS of patients, providing an idea that blockade of the inhibitory receptor or its ligand has the potential to unleash immunity against tumor (Sun, Xu, Huang, et al, 2017, NK Cell Phenotypic Modulation in Lung Cancer Environment, 2021, Gooden, Lampen, Jordanova, et al, 2011, Levy, Bianchini, Von Euw, et al, 2008, (Kren, Slaby, Muckova, et al, 2011) Monalizumab is the humanized NKG2A-targeting monoclonal antibody that is now under investigation for its safety and efficacy. Data from the results of clinical trials about various cancer indication reveals its possibility and reliability as the treatment for solid and hematologic malignancies, especially in combination with other monoclonal antibodies or cancer therapy.

ACKNOWLEDGMENTS

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