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New emerging targets in cancer immunotherapy: the role of LAG3

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ABSTRACT

The success of immunotherapy in many disease entities is limited to a specific subpopulation of patients. To overcome this problem, dual blockade treatments mainly against cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and programmed cell death receptor (ligand) 1 (PD-(L)1) axis were developed. However, due to high toxicity rates and treatment resistance, alternative pathways and novel strategies were desperately needed. Lymphocyte-associated gene 3 (LAG3) represents an inhibitory receptor, which is mainly found on activated immune cells and involved in the exhaustion of T cells in malignant diseases. Its co-expression with other inhibitory receptors, particularly with PD-1 leads to an extensive research on the blockade of LAG3 and PD-1 in preclinical settings. Interestingly, several in-vivo approaches demonstrated a highly significant clinical benefit under dual blockade, whereas the efficacy was very low in case of single agent targeting. Moreover, human tumour tissues showed co-expression of LAG3 and PD-1 in infiltrated lymphocytes, which again generated a rationale for blocking these both molecules in clinical settings. The ongoing clinical studies mainly use dual blockage of LAG3/PD-1, which demonstrated promising survival benefits and long duration of response rates. The following review focuses on the biological background and rationale of combining LAG3 with other agents and serves as an update on the state of clinical research on LAG3 targeting.

BIOLOGICAL BACKGROUND

Lymphocyte-associated gene 3 (LAG3) was identified in the 1990s as a novel transmembrane protein consisting of 489 amino acids with a structural homology to CD4, as both exhibit four extracellular domains.[1] Furthermore, the LAG3 gene is located close to the CD4 gene on chromosome 12. Despite these similarities on chromosomal localisation and similar intron/exon organisation, only approximately 20% of the amino acid sequences of these two molecules were identical. Structural properties were also similar with same extracellular folding patterns, which resulted in binding of LAG3 to major histocompatibility complex (MHC) class II as a ligand, even with an up to 100 times higher affinity than CD4.[2–4]

LAG3 is mainly expressed in activated T and natural killer (NK) cells and was identified to as a marker for the activation of CD4+ and CD8+ T cells.[5] Under pathological conditions, such as chronic inflammation or tumour environment, enhanced LAG3 expression on T cells was observed in combination with other inhibitory receptors such as programmed cell death receptor 1 (PD-1), T cell immunoglobulin and ITIM domain (TIGIT), T cell immunoglobulin-3 (TIM3), CD160, 2B4, which finally led to T cell dysfunction.[6] Furthermore, LAG3 was mainly found on tumour infiltrating regulatory T cells (Tregs) in many types of cancer when compared with non-malignant peripheral cells.[7]

Apart from immune and cancer cells, high LAG3 mRNA expression was commonly found in the red pulp of the spleen, thymic medulla and at the base of the cerebellum.[5]

Modulation of LAG3 expression and its cleavage from the cell surface is an obligatory process for optimal T cell function. Via this cleavage, soluble LAG3 (sLAG3) is released to the circulation, where so far no clear biological function has been identified.[8 9] Despite a lack of clinical evidence, detection of sLAG3 might serve as a prognostic biomarker in tuberculosis and as a diagnostic biomarker in type 1 diabetes.[10 11] From a clinical perspective, sLAG3 might provide information on the activation status of LAG3 and could be used as a biomarker in clinical studies testing new immunotherapies. Recently, patients with metastatic hormone-receptor positive breast cancer receiving immunotherapy had longer disease free and overall survival rates, when sLAG3 was detectable in serum.[12 13]

The co-expression of LAG3 with other inhibitory molecules including PD-1, TIGIT, TIM3, 2B4, CD160 induces the exhaustion of immune cells, which results in diminished cytokine secretion.[14 15] In line with these findings, the blockade of LAG3 on CD4 cells led to elevated production of interleukin (IL)-2, IL-4, interferon gamma and tumour necrosis factor alpha.[16] Earlier studies demonstrated little effect of LAG3 blockade on the resolution of T cell exhaustion, whereas dual LAG3/PD-1 blockade provided very

significant results. Interestingly, both LAG3 and PD-1 deficient mice were usually capable to reduce large tumour volumes, whereas respective single knockouts showed only limited effects.[17] In line with this finding, in colon adenocarcinoma and fibrosarcoma tumour models, anti-PD-1 monotherapy revealed only limited benefits with a tumour clearance of 40% and 20%, respectively, whereas this increased to 80% and 70% when dual blockade with LAG3/PD-1 compounds was used.[17 18] Similar observations were reported in ovarian tumours,[19] melanomas,[20] lymphomas[19] and multiple myelomas.[21] As a hallmark of these in-vivo studies, increased survival and tumour clearance were mainly induced by repair of CD8+ T cell function and elevated cytokine production.

These highly significant in-vivo data demonstrate a clear synergy between LAG3 and PD-1 and accelerate the investigation of these inhibitory receptors on human samples and in further clinical trials as double blockade immunotherapies.

In humans, LAG3 was generally found to be co-expressed with PD-1, which together induced a T cell exhaustion state. Mainly, CD8+ positive tissue infiltrating lymphocytes isolated from patients with hepatocellular carcinoma, ovarian cancer and melanoma showed significant upregulation of LAG3 and high levels of PD-1.[22–25] MHC class II molecules, as ligands of LAG3, are expressed in a variety of cells and tumours such as melanomas.[26] LAG3 was frequently found to be ligated on MHC class II on melanoma cells, which lead to a clonal exhaustion of melanoma infiltrating T cells, thereby avoiding apoptosis.[27] In colorectal cancer, LAG3 was found at higher extent in microsatellite instability high tumours, which are known to be susceptible to immunotherapy.[28] Furthermore, LAG3 expression was found not only in tissue infiltrating lymphocytes but also in peripheral Tregs, tumour involved lymph nodes and within the tumour tissue itself, in melanoma and colon carcinoma.[29] In patients with head and neck squamous cell carcinoma and non-small cell lung cancer, LAG3 was expressed on tumour infiltrating Tregs.[30 31]

TARGETED AGENTS UNDER DEVELOPMENT

Targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and PD1/PD-L1 axis induced clinically relevant objective response rates and extended overall and progression free survival in patients with several different tumour types.[32 33] However, the majority of patients does not respond or develops a treatment resistance after responding to an immunotherapy.[34 35] Additionally, a significant increase of toxicity in patients receiving the dual treatment, particularly with CTLA4 inhibitors, became an important clinical problem.[36] Hence, there exists a clinical need for the identification of more inhibitory receptors, which might contribute to and increase the activity of the up to now identified immunotherapy components. Besides from approaches, which test combination of immunotherapy with chemotherapy, radiation therapy or cancer vaccines, novel combinations focus on the dual immune checkpoint blockade. In this regard, novel inhibitory molecules including LAG3 and TIM3 are gaining more importance as new targets.[15 37] The main goal of dual immunotherapy blockade should be the enhancement of the efficacy by extending progression free and overall survival without increasing the toxicity significantly.

The rationale of targeting LAG3 in addition to PD-1 and the strong preclinical data showing the efficacy of dual blockage of LAG3/PD-1 axis lead to the development of different drugs against the LAG3 molecule. First

Table 1 Compounds developed to target the LAG3 molecule in different biological structures

Name of the compound	Mechanism of action	Phase of clinical trial development	Company
IMP321	APC activator	II	Prima BioMed/Immutep
Relatlimab (BMS986016)	Fully human IgG4 mAb	III	Bristol-Myers Squibb
LAG525	Fully human IgG4 mAb	II	Novartis
MK-4280	Fully human IgG4 mAb	I	Merck
Sym-022	Fully human Fc-inert mAb	I	Symphogen
TSR-033	Fully human IgG4 mAb	I	Tesaro
REGN3767	Fully human mAb	I	Regeneron Pharma/Sanofi
MGD013	DART protein binding both LAG3 and PD1	I	MacroGenics
FS118	Bispecific antibody binding both LAG3 and PD-L1	I	F-Star Delta
INCAGN02385	Fc engineered IgG1k antibody	I	Incyte Biosciences
EOC202	Human LAG-3 fusion protein	I	EddingPharm Oncology

Only compounds, which entered clinical testing, are demonstrated in the table.
APC, antigen presenting cells; DART, dual affinity re-targeting; Ig, immunoglobulin; LAG3, lymphocyte-associated gene 3; PD-1, programmed death receptor 1; PD-L1, programmed death receptor ligand 1; mAb, monoclonal antibody.

Table 2 Current ongoing clinical trials investigating anti-LAG3 drugs in various solid tumours and haematological malignancies as of December 2018

ClinicalTrials.gov identifier (or other database)	Tumour type	Setting (early or advanced disease, first, second or more lines if mts)	Phase	Treatment arms	Target accrual
NCT03252938	Advanced solid tumours	Second line	I	IMP321 (injection directly into the tumour)	38
NCT03625323	Advanced NSCLC Advanced HNSCC	First line or immunotherapy refractory Second line	II	IMP321+pembrolizumab	120
NCT02614833	Met breast cancer, HR positive	First line	II	IMP321	241
NCT02658981	Glioblastoma	Second line	I	Relatlimab±nivolumab	260
NCT02061761	B-Cell malignancies	Relapsed or refractory	I/IIa	Relatlimab±nivolumab	132
NCT01968109	Advanced solid tumours	Second line (melanoma and NSCLC cohorts first line)	I/IIa	Relatlimab±nivolumab	1000
NCT03610711	Advanced gastro-oesophageal tumours	Second line	I	Relatlimab+nivolumab+RTX	15
NCT03044613	Oesophagus and GEJ, stage II/III	First line	I	Relatlimab+nivolumab+RTX	32
NCT02966548	Advanced solid tumours	Second line	I	Relatlimab±nivolumab	45
NCT03662659	Advanced gastric or GEJ	First line	II	Relatlimab+nivolumab	250
NCT03459222	Advanced solid tumours	First line	I/II	Relatlimab+nivolumab+ IDO inhibitor or relatlimab+nivolumab+ipilimumab	230
NCT03607890	MSI-H solid tumours	Refractory to previous PD-(L)1 Treatment	II	Relatlimab+nivolumab	21
NCT02519322	Resectable stage IIIB/IV Melanomas	First line, peri-Operative	II	Relatlimab+nivolumab	53
NCT02060188	MSI-H and non MSI advanced colorectal cancer	First and further line	II	Relatlimab+Nivolumab	340
NCT03470922	Advanced melanoma	First line	II/III	Relatlimab	700
NCT03642067	MSS stable advanced colorectal carcinomas	Second and further line	II	Relatlimab+nivolumab	64
NCT02460224	Advanced solid tumours	Second line	I/II	LAG525±PDR001	515
NCT03365791	Advanced solid tumours and haematological malignancies	Second and further line	II	LAG525+PDR001	160
NCT02720068	Advanced solid tumours	Salvage setting	I	MK-4280±pembrolizumab	408
NCT03489369	Advanced solid tumours or lymphomas	Second line or where no standard treatment available	I	Sym022	30
NCT03250832	Advanced solid tumours	Second line	I	TSR-033±anti-PD-1	260
NCT03005782	Advanced solid tumours and lymphomas	Second line	I	REGN3767±REGN2810	546
NCT03219268	Advanced solid tumours and haematological malignancies	Second line	I	MGD013	243
NCT03440437	Advanced solid tumours and haematological malignancies	After PD-1/PD-L1 blockage	I	FS118	51

Continued

Table 2 Continued

ClinicalTrials.gov identifier (or other database)	Tumour type	Setting (early or advanced disease, first, second or more lines if mts)	Phase	Treatment arms	Target accrual
NCT03538028	Advanced solid tumours and lymphomas	Salvage setting	I	INCAGN02385	55
NCT03600090	Metastatic breast cancer	First line	I	EOC202+CHT	18

CHT, chemotherapy; GEJ, gastro-oesophageal junction; HNSCC, squamous head and neck cancer; HR, hormone receptor; IDO, indoleamine (2,3)-dioxygenase; LAG3, lymphocyte-assoiated gene 3; MSI-H, microsatellite instability high; NSCLC, non-small cell lung cancer; PD-1, programmed death receptor 1; PD-L1, programmed death receptor ligand 1; RTX, radiation therapy.

example was IMP321, which was designed to be a fusion recombinant protein having four extracellular domains like LAG3. This was followed by the development of monoclonal antibodies from various suppliers. Although these drugs are mainly used in clinical settings in combination with anti-PD-1 medication, some anti-LAG3 drugs were generated to be bispecific and target both LAG3 and PD-1, such as FS118 or dual affinity re-targeting protein MGD013.

These compounds, the mechanism of action and the producing company are summarised in table 1.

CURRENT ONGOING CLINICAL TRIALS

The early phase I dose escalation trials of IMP321 as a monotherapy performed at patients with metastatic renal cell carcinoma tested subcutaneous administration of doses raging between 0.05 mg and 30 mg.[38] This was safe and well tolerated. The response rates were however modest and mainly observed in patients with higher doses. This early trial demonstrated the rationale of combining the anti-LAG3 regimen with chemotherapy or other immunotherapies in order to increase the response and survival rates. Based on these results, two further trials in advanced pancreatic cancer and metastatic breast cancer in combination with chemotherapy were designed. Within the first trial, IMP321 was combined with gemcitabine in patients with advanced pancreatic cancer.[39] The efficacy and immunomodulation was insufficient that the authors recommended using higher doses of treatment in further trials. Within the second phase I trial, patients with metastatic breast cancer were treated with IMP321 in combination with paclitaxel in a first-line setting.[40] Patients received different doses of IMP321 via subcutaneous injection every 2 weeks for 6 months. Toxicity was acceptable; there was a significant durable response with enhanced clinical outcome when compared with historical controls. The data of this trial lead to conduction of the placebo-controlled randomised phase II trial, where IMP321 was tested together with paclitaxel in patients with metastatic hormone receptor positive breast cancer. The preliminary efficacy data was demonstrated at American Society of Clinical Oncology (ASCO) congress in 2018.[41] Here, a biweekly dose of 30 mg was recommended for further testing of the phase II trial, since this dose was shown to be the most effective with the least toxicity. IMP321 led to a steady and enhanced antigen-presenting cells (APC) and T cell activation. Within the same congress, the same dosage of IMP321 was demonstrated to be safe in combination with pembrolizumab in patients with melanoma who had a progressive disease after immunotherapy.[42]

BMS-986016 (relatlimab) was the first anti-LAG3 mAb to be developed and is currently being evaluated in various clinical trials including solid tumours as well as haematological malignancies. The initial phase I/IIa trial of relatlimab sought to investigate the efficacy of the drug alone or in combination with nivolumab in patients with advanced diseases. The preliminary data of this trial investigating relatlimab and nivolumab in patients with melanoma who progressed after immunotherapy were demonstrated at ASCO congress in 2017.[43] The efficacy was encouraging with an acceptable safety profile. At the time of the congress report, 31 patients had evaluable results with an ORR of 16% and DOR of 45%.

Further concepts of clinical trials testing other anti-LAG3 regimen were introduced at the latest large oncological congresses, and the data is expected to be published within the next few years. Table 2 summarises the ongoing clinical trials of various anti-LAG3 drugs in different settings. So far, relatlimab represents the only drug, which reached a phase III trial testing and several phase II approaches. Some other drugs including MK-4280, Sym022, TSR-033, REGN3767, MGD013, FS118, INCAGN02385 and EOC202 are in early phase I testing.

CONCLUSIONS

Due to the success gained by the dual blockage of CTLA4 and PD-(L)1, new approaches for single immunotherapies, which might enhance their efficacy are established. In the last few years, LAG3 has gained widespread interest as an inhibitory receptor. Its synergistic biology with PD-1 provided a reasonable rationale to combine this molecule with anti-PD-1 agents. There are currently 11 molecules, which target LAG3 and are already being tested in clinical trials. The results of the early clinical trials demonstrate modest benefit of single anti-LAG3 treatment, which again supports potential combination approaches with other inhibitory receptors.

There are still many unanswered questions regarding the LAG3 biology including its exact function and the existence of ligands other than the MHC class II. Until now, LAG3 has mainly been combined with the PD-(L)1 axis in clinical trials. If a combination with other inhibitory molecules is feasible or not still remains unclear. The clinical implications of LAG3 as a biomarker is also a less reported issue. Whether circulating soluble LAG3 or tissue expression of LAG3 could be used as biomarkers in the course of immunotherapy is now one of the questions, which hopefully will be answered in large clinical trials. Nevertheless, apart from the lack of knowledge on its function and its effector cells, preliminary data, which has been demonstrated so far by phase I trials and congress reports, shows promising and durable response rates with acceptable toxicity, making this molecule a focus of intensive research.

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