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Review

The evolving landscape of 'next-generation' immune checkpoint inhibitors: A review

Luca Mazzarella [a], Bruno Achutti Duso [a], Dario Trapani [a,b], Carmen Belli [a], Paolo D'Amico [a,b], Emanuela Ferraro [a,b], Giulia Viale [a,b], Giuseppe Curigliano [a,b,*]

[a] *New Drugs and Early Drug Development for Innovative Therapies Division, IEO, European Institute of Oncology IRCCS, Milan, Italy*
[b] *University of Milano, Department of Hematology and Hemato-Oncology, Italy*

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TLR, RLR and cGAS/STING

Abstract 'First-generation' immune checkpoint inhibitors targeting Cytotoxic T-Lymphocyte Antigen 4 (CTLA4) and Programmed death-ligand 1 (PD(L)1) have undoubtedly revolutionised the treatment of multiple cancers in the advanced setting. Targeting signalling pathways other than core inhibitory modules may strongly impact the outcome of the antitumour immune response. Drugs targeting these pathways ('next-generation' immune modulators, NGIMs) constitute a major frontier in translational research and have generated unprecedented scientific and financial investment. Here, we systematically reviewed published literature, abstracts from major cancer conferences and pharma pipelines to identify NGIMs that have reached clinical development. We identified 107 molecules targeting 16 pathways, which we classified into 6 groups according to function (inhibitory vs stimulatory) and cell of predominant expression (lymphoid, non-lymphoid and natural killer). We identified all registered past and ongoing clinical trials (n = 428). We summarise the preclinical rationale for these targets, extracting translationally relevant information, and review published and preliminary clinical results. Some targets like indoleamine-2,3-dioxygenase 1, lymphocyte activation gene-3 and IL15 have experienced exceptional growth of interest, measured in terms of activated studies and expected patient enrolment over time. We conclude that in this vast and rapidly changing drug development landscape, novel trial designs and better biomarker identification are necessary to optimise resource allocation

* *Corresponding author:* University of Milano New Drugs and Early Drug Development for Innovative Therapies Division European Institute of Oncology, IRCCS, Via Ripamonti, 435, 20141 Milano, Italy. Fax: +39 02 94379 224.
E-mail address: giuseppe.curigliano@ieo.it (G. Curigliano).

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1. Introduction

Currently approved immune checkpoint inhibitors (ICIs) target core inhibitory pathways (CTLA4 and PD(L)1) that sit atop of an intricate network of opposing signals, evolved to avoid overreaction to self- or non–self-antigens. Despite their disruptive impact, still most patients do not respond or experience major immune-related toxicity, especially when both pathways are co-targeted. Thus, interest has grown towards the numerous co-inhibitory or co-stimulatory molecules, which populate the tumour microenvironment and may dominate the landscape of immunologically refractory tumours. These include signals acting on the final effectors (lymphocytes and natural killer (NK) cells) but also non-lymphoid cells (innate immune system, fibroblasts, endothelium or tumour cells themselves) [1]. The emergent panoply of 'next-generation' immune modulators (NGIMs) has contributed to the dramatic growth of the immuno-oncology field, with an astonishing ~52.000 predicted enrolments from 469 trials with dozens of new compounds in 2017 [2].

Here, we reviewed the published literature, major conference proceedings and pharma pipelines, identifying molecules developed or in development in solid tumours. We omitted more complex agents like cell therapy, vaccines, oncolytic viruses and so on and those exclusively investigated in haematological malignances. Data included in the main figures only concern molecules that have reached clinical development; those at preclinical stage of development are included in the supplementary table.

To identify NGIMs, we searched abstracts of recent major cancer conferences (American Society of Clinical Oncology (ASCO), American Association for Cancer Research (AACR) and Society of Immunotherapy of Cancer (SITC)) and publicly available pharma pipelines for immunotherapy (IO) drugs. We identified 107 molecules currently in clinical development (plus additional compounds in preclinical development). Drugs were assigned to 16 pathways, grouped according to function and cell of predominant expression: lymphoid inhibitors (lymphocyte activation gene-3 [LAG3], transmembrane immunoglobulin and mucin domain 3 [TIM3], T cell immunoglobulin and ITIM domain [TIGIT], adenosine pathway), lymphoid stimulators (OX40, glucocorticoid-induced tumour necrosis factor receptor [TNFR]–related [GITR], 41BB, inducible co-stimulator [ICOS]), non-lymphoid inhibitors (indoleamine-2,3-dioxygenase 1 [IDO1], colony-stimulating factor-1 [CSF1]/CSF1R, transforming growth factor beta [TGFb], CD47/SIRPa, chemokines), non-lymphoid stimulators (pathogen-associated molecular pattern [PAMP]/damage-associated molecular pattern [DAMP] receptors and CD40) and NK stimulators (KIR2R and IL15) and inhibitors (NKG2A) (Fig. 1). We systematically identified all associated trials in clinicaltrials.gov using the package *rclinicaltrials* in R (see supplementary methods). We calculated the total trial number and expected enrolled patients (actual enrolment not available). All trials and molecules and additional references are reported in supplementary information.

2. The NGIM drug development landscape: an overview

We identified 428 trials involving NGIMs with a projected enrolment population of 51,714 patients since 2005 (Figs. 2–4). Selected trials are summarised in Table 1. Analysis of enrolment over time (Fig. 3) shows the rapid surge of interest in non-lymphoid inhibitors with a sharp acceleration after 2014, with >25 k expected enrolled patients. A large share of the clinical development is taking place in phase 1/2, with very few agents already progressed to phase 3 (Fig. 2). Peculiar is the case of IDO1, a target at the centre of enormous commercial interest by pharmaceutical companies recently [3] with 10 planned phase 3 studies with >15 k planned enrolments. The field was strongly impacted by the recent failure of the ECHO-301 with epacadostat in melanoma causing the termination or 'downgrading' to phase 2 of many other programs (see below). Interest in targeting LAG3 and NK cells has surged more recently with the activation of several phase 2/3 trials after promising initial results. Toll-like receptor (TLR) and CSF1 agonists, already investigated in the mid-2000s, are experiencing renewed interest, especially in combination with ICIs. Other intriguing combinations are emerging based on preclinical data, with immunotherapeutics (e.g. OX40 and TLR agonists, CD40 agonists with CSF1R antagonists) or with other modalities like radiotherapy, chemotherapy or antibody-mediated targeted therapy.

Specific drug classes are analysed more in detail in the following section.

3. Lymphoid inhibitors

Targets in this group are often co-expressed with PD1/CTLA4 but play a more nuanced role, sometimes within anatomically defined districts [4]. Consequently, agents are often ineffective alone and usually explored with PD(L)1/CTLA4 blockade, to which they apparently add little toxicity.

3.1. Lymphocyte activation gene-3

Initially considered a competitive MHC II inhibitor due to CD4 homology [5], LAG3/CD223 is now known to elicit more widespread inhibition through interaction with several other ligands, of which the best characterised is LSECtin, a lectin selectively expressed in the liver and in melanoma cells that may confer a particular

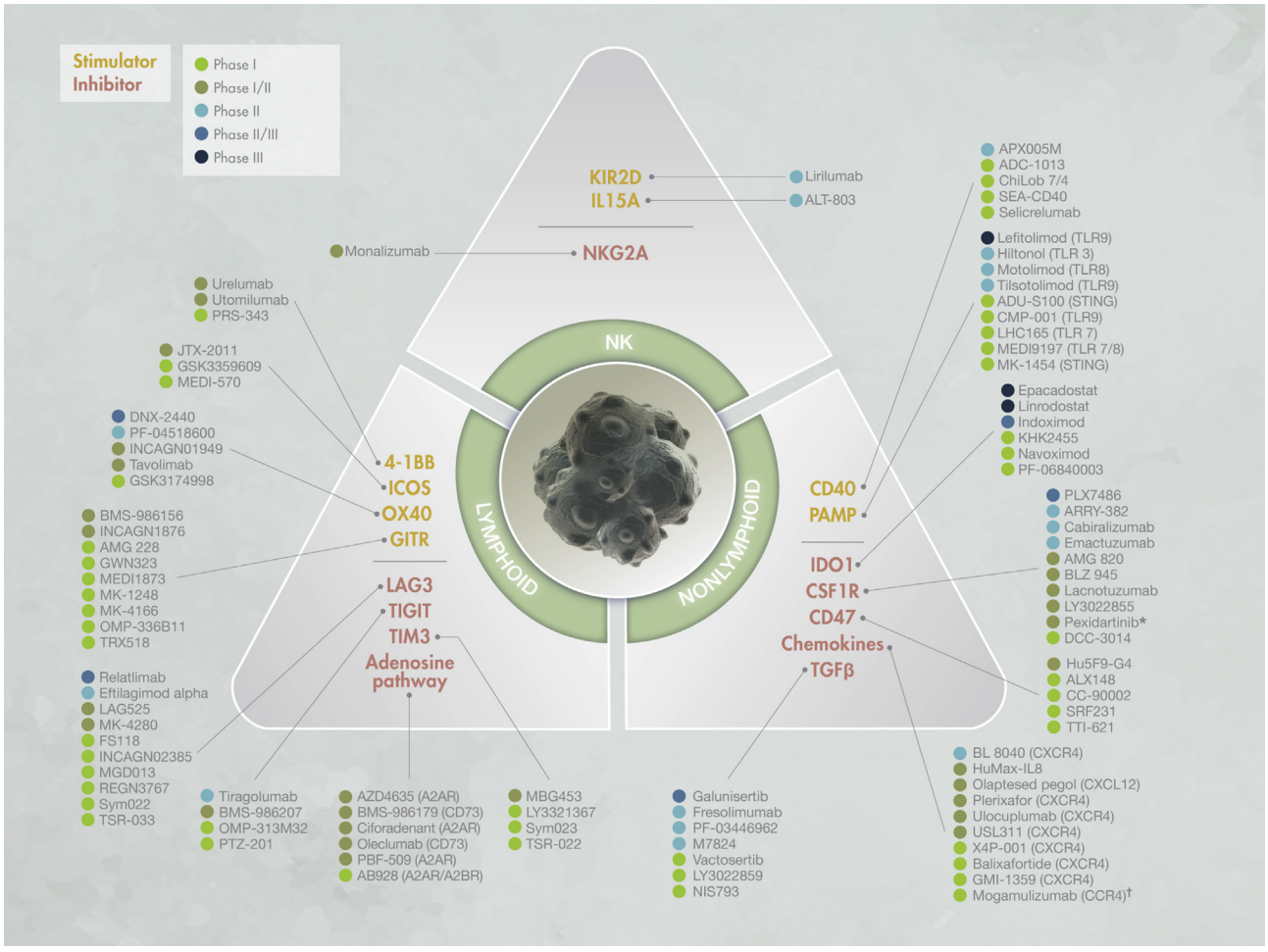

Fig. 1. **Grouping of drugs by immune-modulating pathway.** Colour code indicates most advanced stage of development. NB: some IDO1 trials recently 'downgraded' to phase 2. IDO1, indoleamine-2,3-dioxygenase 1; ICOS, inducible co-stimulator; GITR, glucocorticoid-induced TNFR-related; TNFR, tumour necrosis factor receptor; LAG3, lymphocyte activation gene-3; TIGIT, T cell immunoglobulin and ITIM domain; TIM3, transmembrane immunoglobulin and mucin domain 3; PAMP, pathogen-associated molecular pattern; TGFβ, transforming growth factor beta.

anatomical tropism to anti-LAG3 responses [6]. LAG3 downmodulates lymphocyte responses in tolerance, chronic infections and cancer, but its genetic or pharmacological deficiency is ineffective alone and requires cooperation with other signals, such as PD1 inhibition [7]. Of the four LAG3 blockers in development, the IgG4 relatlimab is the most advanced. In combination with nivolumab, it showed efficacy (11.5% overall response rate [ORR] and 49% disease control rate [DCR]) in ICI-pretreated melanoma patients, a significant result for a patient population with no solid therapeutic option to date. Responses were related to LAG3 expression, and toxicity was not superior to historical controls with nivolumab alone [8]. These results, if confirmed on larger numbers, would make LAG3 a promising target in the melanoma field.

Other agents include the IgG4 MK-4280 and IMP701, tested with anti-PD1 in multiple tumours, and IMP321/eftilagimod, a recombinant soluble LAG3-Ig fusion protein, tested with paclitaxel in breast cancer (AIPAC [9]) and with pembrolizumab in melanoma (TACTI-mel, ref SITC)

3.2. Adenosine pathway

Signalling through bioactive adenosine derivatives exerts a profound activity on the immune environment. Hypoxia and TGFb are key determinants of bioactive adenosine production and provide an intriguing link between tumour metabolism and immune suppression [10]. Hypoxia induces release of ATP or NAD+, which becomes a possible source of bioactive adenosine through the activity of multiple enzymes expressed on the surface of cancer cells (ectonucleotidases CD39 and CD73, alkaline phosphatase, CD38, CD203a), which are then sensed by immune cells, especially T and NK cells, by their amine receptors (cAMP-modulating receptors, of which the cAMP-increasing A2AR and A2BR play

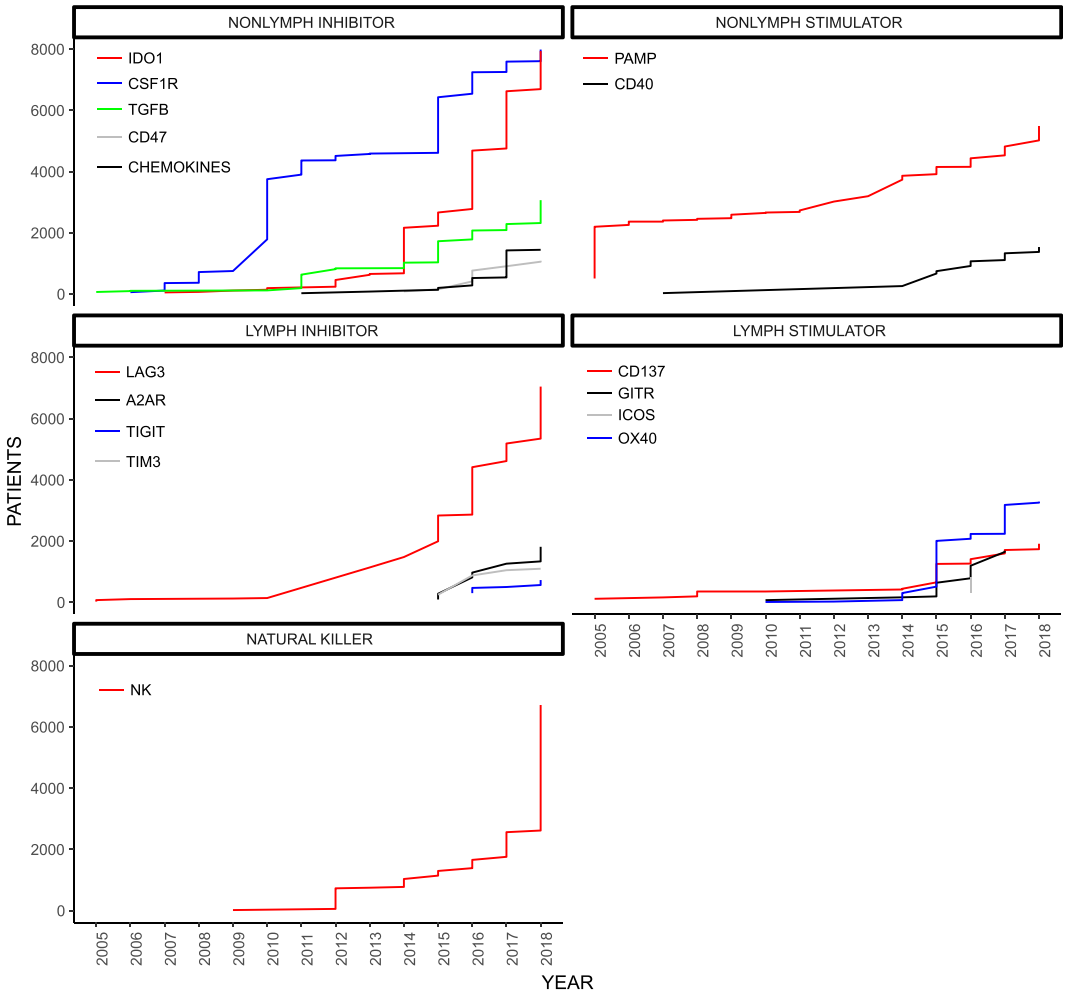

Fig. 2. **Cumulative increase in the number of projected enrolled patients since 2005.** All molecules within each pathway contribute to the sum. PAMP, pathogen-associated molecular pattern; IDO1, indoleamine-2,3-dioxygenase 1; TGFB, transforming growth factor beta; GITR, glucocorticoid-induced TNFR-related; TNFR, tumour necrosis factor receptor; ICOS, inducible co-stimulator; LAG3, lymphocyte activation gene-3; TIGIT, T cell immunoglobulin and ITIM domain; TIM3, transmembrane immunoglobulin and mucin domain 3; NK, natural killer.

the main role) [10]. CD73 and A2AR are the targets most explored pharmacologically. Ablating either one improves the activity of cytotoxic lymphocytes and reduces tumour growth, but ablating both is required for significant survival advantage in some preclinical models [11] Synergy with anti-PD1 or anti-PDL1 and anti-CTLA4 therapies is supported by preclinical studies [12–14].

CD73 was also recently found to be a prognostic factor in multiple tumours [15]. Targeting molecules include monoclonal antibodies that inhibit enzymatic activity and/or promote CD73 internalisation. Agents in clinical development, alone or with anti-PD1/CTLA4, include MEDI9447 (oleclumab), an IgG1 [12], and BMS-986179, an IgG2-IgG1 hybrid with an engineered Fc sequence. A phase I first-in-human study with oleclumab alone or in combination with durvalumab in patients with advanced pancreatic or microsatellite stability-colorectal cancer showed a manageable safety profile and encouraging clinical activity for the combination arm [16]. Small molecules are also in preclinical development.

Agents targeting A2AR are orally available small molecules that competitively inhibit adenosine binding. CPI-444 is the first in class, originally investigated as a potential new treatment for Parkinson disease due to adenosine receptor involvement in dopaminergic system [17]. Preliminary phase 1 results, tested alone or with atezolizumab, showed good safety but relatively disappointing ORR [18]. Other agents of the same class, still at early stages of development, include AZD4635 and PBF-509/NIR178, with studies focussing particularly on lung cancer (Morpheus Lung/NCT03337698, AdenONCO/NCT02403193). NIR178 recently showed a favourable safety profile with promising clinical benefit in 24 patients with advanced non–small-cell lung cancer

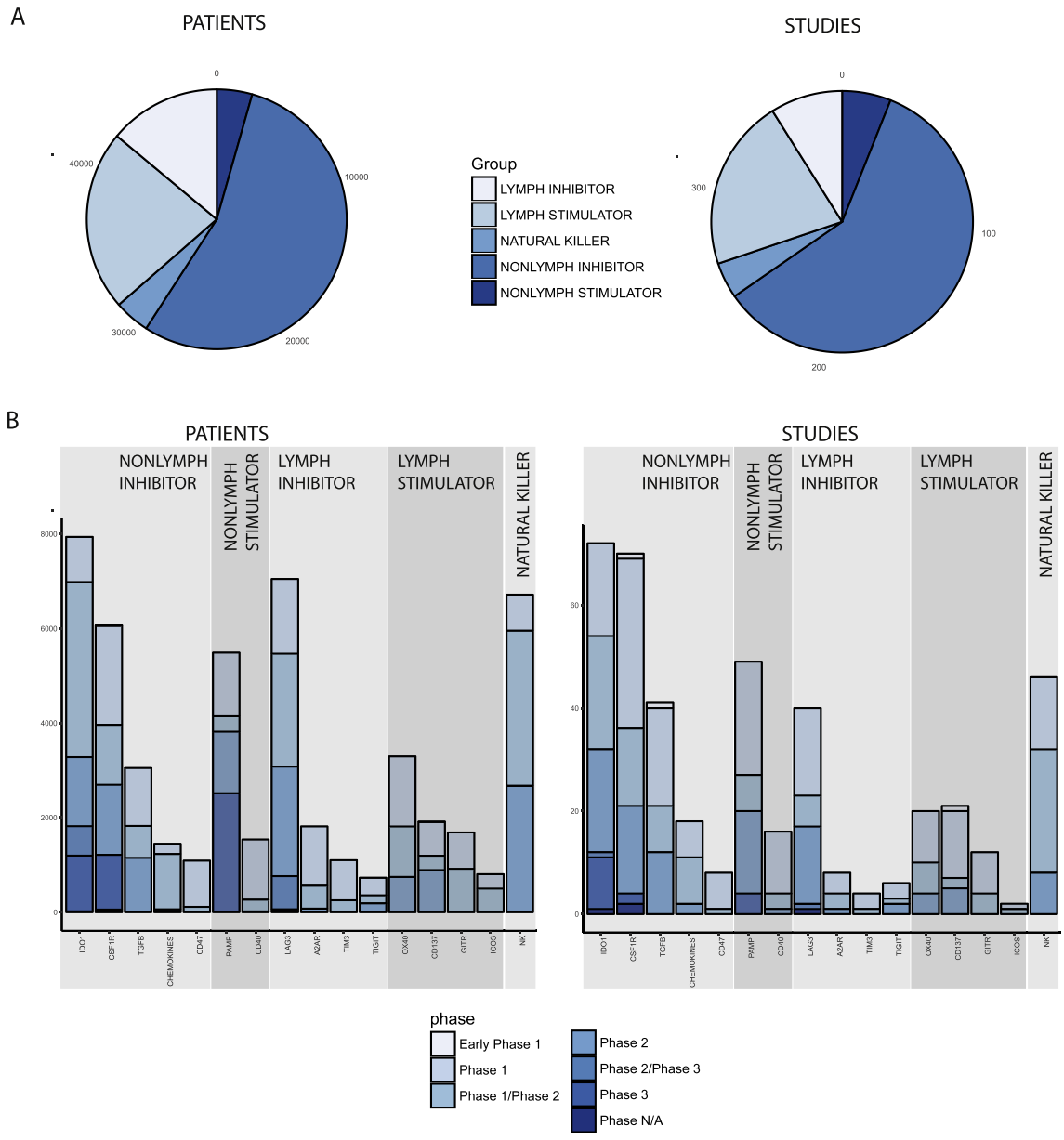

Fig. 3. **Total number of active or completed trials and the associated projected enrolled patients.** Trials or patient breakdown by drug class (A) or by trial phase (B).

(NSCLC) either previously IO exposed or IO naïve, irrespective of PD-L1 status (AdenONCO/NCT02403193). More A2AR inhibitors are under pre-clinical development, including EVOEX21546 and AB928 (a dual antagonist of the A2AR and A2BR adenosine receptors).

3.3. T cell immunoglobulin and ITIM domain

TIGIT is an Ig-like protein homologous to CD28, which interferes with a recently discovered co-stimulatory axis reminiscent of the CTLA4/B7/CD28 pathway, consisting of the CD226 receptor (on NK, T and monocytes) and ligands CD155 and CD122, on Antigen Presenting Cells (APCs), T cells and stromal and tumour cells [4]. Intriguingly, the bacterium *Fusobacterium nucleatum*, an inducer of chemoresistance [19], directly engages TIGIT [20], suggesting a precise mechanism for the role of microbiota in tumour immunity. TIGIT plays a key role in autoimmunity and chronic viral infections and promotes Treg-mediated suppression, especially of Th1/Th17 responses (many refs); TIGIT-expressing cells show exquisite tropism for the tumour microenvironment. TIGIT inhibition synergises with PD1 and, intriguingly, TIM3 [21,22]. Three molecules are in clinical development to date, alone or in combination with anti-PD(L)1 agents (tiragolumab, BMS-986207 and OMP-313M32), with no available clinical data.

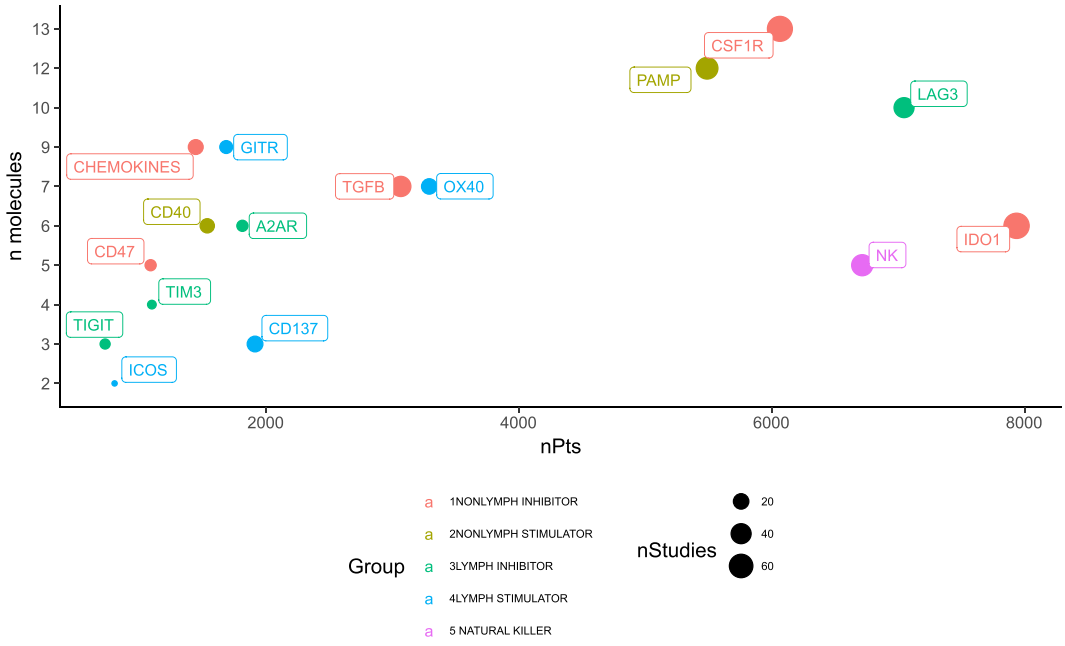

Fig. 4. **Summary of the NGIM development landscape.** Each pathway is graphed according to the number of molecules in clinical development (x axis), cumulative sum of expected enrolled patients (y axis) and number of studies, including both open and closed (bubble size). Each pathway is colour-coded according to the group. NGIM, next-generation immune modulator; GITR, glucocorticoid-induced TNFR-related; TNFR, tumour necrosis factor receptor; TGFB, transforming growth factor beta; TIM3, transmembrane immunoglobulin and mucin domain 3; TIGIT, T cell immunoglobulin and ITIM domain; ICOS, inducible co-stimulator; PAMP, pathogen-associated molecular pattern; LAG3, lymphocyte activation gene-3; NK, natural killer; IDO1, indoleamine-2,3-dioxygenase 1.

3.4. Transmembrane immunoglobulin and mucin domain 3

TIM3 is a glycoprotein with poorly understood signalling mediators and multiple and biochemically heterogeneous ligands, including most prominently the immunosuppressive carbohydrate-binding protein galectin 9 [23,24] but also phosphatidylserine [25], the immune-stimulatory DNA alarmin HMGB1, for which TIM-3 may act as a 'sink' [24] and most recently, the carcinoembryonic antigen–related cell adhesion molecule 1 [23]. TIM3 is often co-expressed with PD1 in exhausted CD8 cells in infections and tumours but also acts in innate cells through a galectin-9–independent, TLR-dependent mechanism [26]. Its blockade in preclinical models is poorly effective alone but synergises with anti-PD1 [27]. Important for clinical translation, TIM3 inhibition exacerbates bleomycin-induced lung fibrosis in mice [28]. Four monoclonal antibodies have been taken to clinical development to date, with as yet no preliminary clinical results: Sym023, TSR-022, MBG453 and LY3321367.

4. Lymphoid stimulators

Four main inducible T cell co-stimulators have been the target of extensive pharmacological research: 4-1BB, GITR, OX40 and ICOS. The first three are structurally related as members of the TNFR superfamily [29]. Design of agonists is complicated by the geometric constraints imposed by the peculiar structure of these receptors, which require multimerisation for productive engagement. Thus, the cross-linking properties of targeting antibodies, influenced by their interaction with Fcg receptors on surrounding cells, may be key for efficacy [30] and synthetic ligands may be more effective than antibodies. A further complication is the seemingly contradictory role played by ICOS, GITR and OX40, all inducibly expressed on T effectors but also constitutively expressed at even higher levels in regulatory T cells. This differential expression allows designing dual-activity agents that productively engage effectors while selectively depleting Tregs, via antibody-dependent cellular cytotoxicity (ADCC). Thus, such agents may require sufficient density of ADCC-competent myeloid/NK cells in the environment, possibly synergising with strategies that enhance innate infiltrate (such as 41BB agonists) [31]. 4-1BB is a 'cleaner' stimulator with a clear Treg-inhibiting activity. Unlike non-PD1/CTLA4 lymphoid antagonists, engagement of these molecules can also be effective as monotherapy [32].

4.1. Inducible co-stimulator

ICOS/CD278 is structurally and functionally similar to CD28, and its biology is closely intertwined with CTLA4, as it is required for and upregulated by CTLA4 blockade [33,34]; ICOS agonists are effective alone [35] but strongly synergise with CTLA4 blockade in poorly immunogenic tumours [36]. ICOS signalling favours

Table 1
Selected studies with NGIM.

Group	Target	NCT ID	Phase	Patients analysed (*n*)	Primary condition (*n*)	Lead sponsor/agency	Non-proprietary name (molecule)	Treatment	Significant adverse events (AEs)/treatment-related AEs (TRAEs) ≥G3 (%)	Efficacy - response rate	Efficacy - time to event	Reference
NK stimulator	KIR2D	NCT01714739	I/II	41	SCCHN	Bristol-Myers Squibb	Lirilumab (IPH2102)	Lirilumab/ nivolumab	No ≥ G3 TRAEs and no DLT, with discontinuation rate of 3%	• ORR 24% (CR 10.3%, PR 13.8%) • DCR 52%	• OS 90% at 6 mo and 60% at 12 mo	J Immunother Cancer 2016; 4(Suppl 2): 91
NK inhibitor	NKG2A	NCT02643550	I/II	17	SCCHN	Innate Pharma	Monalizumab (IPH2201)	Monalizumab/ cetuximab	Fatigue (6)	N/A	N/A	AACR; Cancer Res 2017;77(13 Suppl):Abstract nr 5666
NK stimulator	IL15A	NCT02523469	I/II	23	NSCLC	Altor BioScience	ALT-803	ALT-803/ nivolumab	Lymphocytopenia G3 (9.5), fatigue G3 (9.5). No G4-5 TRAEs	N/A		[127]
Lymphoid stimulator	4-1BB	NCT01307267	I	55	Advanced neuroendocrine (Merkel cell) carcinoma of the skin (15), CRC (12), gastric cancer (4), pancreatic cancer (4), lung cancer (3), hepatobiliary cancer (3), BC, lymphoma, soft tissue sarcoma (2 each) and 8 other tumours	Pfizer	Utomilumab (PF-05082566)	Utomilumab	Overall G3/4 AEs (32.7), in a single patient	• ORR (solid tumours) 3.8%	• Median PFS 1.7 (95% CI, 1.6−1.8) mo • Median OS 11.2 (95% CI, 6.1−24.1) mo for all solid tumours	Clin Cancer Res 2018; 24(8):1816−1823
Lymphoid stimulator	4-1BB	NCT02179918	I	23	Advanced solid tumours	Pfizer	Utomilumab (PF-05082566)	Utomilumab/ pembrolizumab	Adrenal insufficiency (4.3), hypokalaemia (4.3), fatigue (4.3), anaemia (13), hyponatraemia (13)	• ORR 26.1%	N/A	Clin Cancer Res 2017; 23(18):5349-5357
Lymphoid stimulator	4-1BB	NCT02253992	I/II	251	Advanced solid tumours, advanced NHL (urelumab single agent); advanced solid tumours, DLBCL, melanoma, NSCLC and SCCHN (urelumab/nivolumab)	Bristol-Myers Squibb	Urelumab	Urelumab Urelumab/ nivolumab	N/A (17)	Monotherapy: • ORR 10%, all in DLBCL • DCR 28%, 18%, 20% and 39% for DLBCL, CRC, SCCHN and other solid tumours, respectively; Combination: • ORR 50% vs 10% • DCR 70% vs 28, 21%, 21%, 35%, 23% and 33% for IO-naïve melanoma; DLBCL, NSCLC progressing to IO, IO-naïve NSCLC, SCCHN and other solid tumours, respectively	N/A	P T 2017 Jan; 42(1): 49 −53
Lymphoid stimulator	ICOS	NCT02904226	I/II	164	Gastric cancer and TNBC	Jounce Therapeutics, Inc.	JTX-2011	JTX-2011 JTX-2011/ nivolumab	TRAEs G3-4 (8 in monotherapy, 13 in combo)	Monotherapy: • PR 1/7 in gastric cancer • SD 2/5 in TNBC Combo: • PR 2 (0.1, 0.3 mg/kg) • SD 2/19 in gastric cancer • PR 1/15 in TNBC	N/A	J Clin Oncol 36, 2018 (suppl; abstr 3000)
Lymphoid stimulator	OX40	NCT02315066	I	48	Melanoma (14), HCC (19), SCCHN (6), RCC (9)	Pfizer	PF-04518600 (PF-8600)	PF-04518600 (PF-8600) PF-04518600/ utomilumab	Fatigue (27.1), nausea and vomiting (8.3)	• ORR 0% • DCR 52% (intended as BOR; if intended as SD > 24 w, DCR 6%)	N/A	J Clin Oncol 2017 35:15_suppl, 3027-3027
Lymphoid stimulator	GITR	NCT02598960	I/II	66	Advanced solid tumours	Bristol-Myers Squibb	BMS-986156	BMS-986156 BMS-986156/ nivolumab	Total (6), all in the combination arm; lipase increase (1.5), lung infection (1.5), fatigue (1), AST increase (1.5) CPK increase (1.5), with discontinuation rate of 1.5%; No DLTs were reported	ORR 37% *for the combination*	N/A	J Clin Oncol 017 35:15_suppl, 104-104
Lymphoid inhibitor	LAG3	NCT01968109	I/II	68	Melanoma which progressed on anti-PD-1/PD-L1	Bristol-Myers Squibb	Relatlimab (BMS-986016)	Relatlimab/ nivolumab	Total (4.4), with a discontinuation rate of 1.5%	• ORR 11.5% • DCR 49% (1 CR); enhanced responses correlated with LAG-3 expression, irrespective of PD-L1 expression	N/A	Ann Oncol 28, mdx440.011 (2017)

Lymphoid inhibitor	LAG3	NCT02614833	II	15	Hormone receptor (HR)-positive BC	Immutep Ltd	Eftilagimod alpha (IMP321)	Eftilagimod alpha/ paclitaxel	N/A (27)	• DCR 87% (PR 47%)	N/A	J Clin Oncol 36, 2018 (suppl; abstr 1050)
Lymphoid inhibitor	LAG3	NCT02676869	I	18	Melanoma progressing to pembrolizumab	Immutep Ltd	Eftilagimod alpha (IMP321)	Eftilagimod alpha/ pembrolizumab	N/A (44)	• DCR 50%	N/A	J Clin Oncol 36, 2018 (suppl; abstr e15099)
Lymphoid inhibitor	A2AR	NCT02655822	I	34	Anti-PD1/PDL1 treatment-refractory RCC and NSCLC	Corvus Pharmaceuticals, Inc./Genentech	CPI-444	CPI-444 CPI-444/ atezolizumab	Tachycardia (N/A)	• DCR 86% and 50% (100% and 43% if PD-1/PD-L1 refractory/ resistant) for RCC and NSCLC, respectively	NA	J Clin Oncol 2017 35:15_suppl, 3004-3004
Non-lymphoid stimulator	TLR8	NCT01836029	II	195	SCCHN	VentiRx Pharmaceuticals	Motolimod (VTX-2337)	Motolimod/ EXTREME (platinum-based chemotherapy, 5-FU and cetuximab) Placebo/ EXTREME	Neutropenia (39.3 vs 36), anaemia (24.7 vs 15.1), stomatitis (7.9 vs 15.1), dermatitis acneiform (7.9 vs 5.8), leucopenia (19.1 vs 17.4), nausea (4.5 vs 2.3), diarrhoea (5.6 vs 3.5), hypokalaemia (4.5 vs 10.5), pyrexia (2.2 vs 0), rash (2.2 vs 1.2), chills (1.1 vs 0), injection site reactions (2.2 vs 0) for motolimod/ EXTREME vs Placebo/ EXTREME, respectively	• ORR 38 vs 34% (p = 0.54) for motolimod/EXTREME vs placebo/EXTREME, respectively	• Median PFS 6.1 vs 5.9 mo (HR 0.99; 1-sided 90% CI, 0.00 −1.22; p = 0.47), • Median OS 13.5 vs 11.3 mo (HR 0.95; 1-sided 90% CI, 0.00 −1.22; p = 0.40) for motolimod/ EXTREME vs placebo/EXTREME, respectively; HPV-positive subgroup analysis: • PFS 7.8 vs 5.9 mo (HR 0.58; 1-sided 90% CI, 0.00−0.90; p = 0.046) • Median OS 15.2 vs 12.6 mo (HR 0.41; 1-sided 90% CI, 0.00 −0.77; p = 0.03)	JAMA Oncol Published online June 21, 2018. https://doi.org/10.1001/jamaoncol.2018.1888
Non-lymphoid stimulator	TLR8	NCT01666444	II	297	Recurrent ovarian, fallopian tube or primary peritoneal carcinoma	VentiRx Pharmaceuticals	Motolimod (VTX-2337)	Motolimod/ pegylated liposomal doxorubicin (PLD) Placebo/PLD	Total AEs ≥ G3 (63.9 vs 61.9), ≥G4 (6.8 vs 9.5) and G5 (4.8 vs 4.1)	• ORR (20.9 vs 21.5%, p = 0.875) • DCR (57.4 vs 61.1%, p = 0.506),	• OS 18.1 vs 18.9 mo (HR 1.22, p = 0.923) • iPFS 4.8 vs 5.2 mo,	Ann Oncol 2017 May 1;28(5):996-1004
Non-lymphoid stimulator	TLR9	NCT02680184	I	68	Advanced melanoma resistant to anti-PD-1 (monotherapy or in combination)	Checkmate Pharmaceuticals	CMP-001	Intratumoral CMP-001/pembrolizumab	Hypotension (10.2), anaemia (2.9), chills (2.9), hypertension (2.9) and fever (2.9)	• ORR 22.5%, 33.3% and 7.7% in all dose cohorts, weekly and q3w, respectively	N/A	Cancer Res 2018 (78) (13 Supplement) CT144
Non-lymphoid inhibitor	IDO1	NCT00567931	I	48	Solid tumours (sarcoma, NSCLC, CRC, melanoma, BC, ovarian cancer, pancreatic cancer, CUP, oesophageal cancer, cervical cancer, uterine cancer, HCC, osteosarcoma, prostate cancer)	NewLink Genetics Corporation	Indoximod (1-methyl-D-tryptophan)	Indoximod	Fatigue (4), anaemia (6), lymphopenia (6); dose-limiting hypophysitis (6)	• DCR 7% (all SD)	N/A	Oncotarget 2016; 7(16): 22,928−22938
Non-lymphoid inhibitor	IDO1	NCT02073123	II	85	Melanoma IO-naïve, cutaneous or mucosal	NewLink Genetics Corporation	Indoximod (1-methyl-D-tryptophan)	Indoximod/ ipilimumab Indoximod/ nivolumab Indoximod/ pembrolizumab	Fatigue (2), diarrhoea (2), rash (2), arthritis (1), gastritis (1), hearing impairment (1); G2 interstitial nephritis - SAE (1)	• ORR 56% (CR 19%)	• Median PFS 12.4 mo	J Clin Oncol 36, 2018 (suppl; abstr 9512)
Non-lymphoid inhibitor	IDO1	NCT01195311	I	52	Solid tumours; CRC (29)	Incyte Corporation	Epacadostat (INCB024360)	Epacadostat (INCB024360)	Transaminitis (4), not related to IO, with drug discontinuation rate of 13.5%	• ORR 0% • DCR 35%	N/A	Clin Cancer Res 2017; 23(13):3269-3276
Non-lymphoid inhibitor	IDO1	NCT02327078	I/II	50	Melanoma	Incyte Corporation	Epacadostat (INCB024360)	Epacadostat/ nivolumab	Rash (19), ALT increase (12), pneumonitis (13%), with discontinuation rate of 16%	• ORR 62% (CR 18%) • DCR 78%	• PFS 63% at 1 y • OS 92% at 1 y	J Clin Oncol 36, 2018 (suppl; abstr 9511)

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Table 1 (*continued*)

Group	Target	NCT ID	Phase	Patients analysed (*n*)	Primary condition (*n*)	Lead sponsor/agency	Non-proprietary name (molecule)	Treatment	Significant adverse events (AEs)/treatment-related AEs (TRAEs) ≥G3 (%)	Efficacy - response rate	Efficacy - time to event	Reference
Non-lymphoid inhibitor	IDO1	NCT02752074	III	706	Melanoma IO naïve	Incyte Corporation; Merck Sharp & Dohme Corp	Epacadostat (INCB024360)	Epacadostat/ pembrolizumab Pembrolizumab	Total G ≥ 3 TRAEs combination (21.8) vs pembrolizumab monotherapy (17)	• ORR 34.2% vs 31.5% in the combination vs pembrolizumab monotherapy, respectively	• Median PFS for combination vs pembrolizumab monotherapy 4.7 vs 4.9 mo, respectively; PFS at 12 mo 37% in both groups (consistent across PD-L1 and BRAF subgroups) • OS not expected to reach statistical significance; OS rate at 12 mo 74% in both groups	J Clin Oncol 2018 36:15_suppl, 108-108
Non-lymphoid inhibitor	IDO1	NCT02658890	I/II	434	Solid tumours; bladder cancer (29)	Bristol-Myers Squibb	BMS-986205, ONO-7701, f001287	BMS-986205/ nivolumab	Total ≥ G3 TRAEs (51); myocarditis G5 (0.2)	Bladder cancer: • ORR 34% (CR 3%) • DCR 48%	N/A	J Clin Oncol 36, 2018 (suppl; abstr 4512)
Non-lymphoid inhibitor	CSF1R	NCT01494688	I	29	Diffuse-type tenosynovial giant cell tumour of soft tissue (pigmented villonodular synovitis)	Hoffmann-La Roche	Emactuzumab (RG7155, RO5509554)	Emactuzumab	Periorbital oedema (4), subacute cutaneous lupus erythematosus (4), dermohypodermitis (4)	ORR 86%, CR 7% DCR N/A (no SD reported)	N/A	Lancet Oncol 2015 Aug;16(8):949-56
Non-lymphoid inhibitor	CSF1R	NCT02807844	I/II	50	Solid tumours (melanoma, endometrial cancer, pancreatic cancer, TNBC)	Novartis Pharmaceuticals	Lacnotuzumab (MCS110)	Lacnotuzumab/ PDR001 (spartalizumab)	AST increase (12), asthenia (10), hyponatremia (10), creatine phosphokinase (6)	• ORR 2% (CR 0%) • DCR 21% (27% according to iRECIST)	N/A	J Clin Oncol 2018 36:15_suppl, 3014-3014
Non-lymphoid inhibitor	CSF1R	NCT02526017	I	205	Advanced solid tumours	Five Prime Therapeutics, Inc.	Cabiralizumab (FPA008)	Cabiralizumab/ nivolumab	Total G3−5 TRAEs attributed to cabiralizumab (43), with discontinuation rate of 13%, CPK elevations (14), AST elevation (5)	Prior CT-treated/IO-naïve pancreatic cancer cohort: • PR 9.6% (MSS) • Prolonged SD 1/31 (182 days) • 1/31 beyond PD with >40% reduction in baseline target lesions (247 days on study) • DCR at 6 mo 13% • ORR 10%	N/A	J Immunother Cancer 2017; 5 (Suppl 3):89
Non-lymphoid inhibitor	CD40	NCT00607048	I	34	Advanced solid tumours	Hoffmann-La Roche	CP-870,893	CP-870,893/ paclitaxel/ carboplatin	Neutropenia (28.1), anaemia (6.2), arrhythmia (6.2), ALT increase (3.1), cytokine release syndrome (3.1), thrombocytopenia (3.1), fatigue (3.1)	• PR 20% • SD 40%	N/A	Oncoimmunology 2013; 2(1): e23033
Non-lymphoid inhibitor	CD40	NCT02376699	I	48	Advanced solid tumours	Seattle Genetics, Inc.	SEA-CD40	SEA-CD40 SEA-CD40/ pembrolizumab	N/A	• DCR 32%	N/A	J Clin Oncol 36, 2018 (suppl; abstr 3093)
Non-lymphoid inhibitor	TGFB	NCT01682187	I	65	Solid tumours; glioma (58)	Eli Lilly and Company	Galunisertib (LY2157299)	Galunisertib/ lomustine	Total G3-4 AEs (40); thrombocytopenia (2)	• ORR 10% (CR 2%) • DCR 18%	N/A	Clin Cancer Res 2015 Feb 1;21(3):553-60
Non-lymphoid inhibitor	TGFB	NCT01582269	II	158	Recurrent GMB	Eli Lilly and Company	Galunisertib (LY2157299)	Galunisertib/ lomustine	Total ≥ G3 TRAEs (22), SAEs (42), TR-SAEs (7)	•ORR 1% (CR 1%) • DCR 21%	• Median OS 6.7 mo • Median PFS 2 mo	Neuro Oncol 2016 Aug;18(8):1146-56
Non-lymphoid inhibitor	TGFB	NCT02517398	I	19	Solid tumours	EMD Serono Research & Development Institute, Inc.	MSB0011359C (M7824)	MSB0011359C	Skin infection secondary to localised bullous pemphigoid (5), asymptomatic lipase increase (5), colitis (5), gastroparesis (5)	• ORR 16% (CR 5%) • DCR 32%	N/A	Clin Cancer Res 2018 Mar 15;24(6):1287-1295
Non-lymphoid inhibitor	TGFB	NCT02517398	I	80	NSCLC IO naïve	EMD Serono Research & Development Institute, Inc.	MSB0011359C (M7824)	MSB0011359C	Total TRAEs ≥ G3 (25) with a discontinuation rate of 8%	• ORR 25% (41−74% in PD-L1 high NSCLC)	N/A	J Clin Oncol 36, 2018 (suppl; abstr 3007)
Non-lymphoid inhibitor	TGFB	NCT02517398	I	16	HPV-associated tumours; cervical (9) anal (4), SCCHN (3)	EMD Serono Research & Development Institute, Inc.	MSB0011359C (M7824)	MSB0011359C	Colitis (6), cystitis (6), gastroparesis (6)	• ORR 38% (CR 6%) • DCR 56%	N/A	J Clin Oncol 36, 2018 (suppl; abstr 9017)
Non-lymphoid inhibitor	CCR4	NCT02476123	I	96	Advanced solid tumours	Kyowa Hakko Kirin Pharma, Inc.	Mogamulizumab (KW-0761)	Mogamulizumab (KW-0761)	Total G3-4 TRAEs (26)	• ORR 26.7% (PR 20%, all for HCC) • SD 33% for pancreatic cancer	N/A	Ann Oncol 28 (Supplement 5): v1−v21, 2017

Non-lymphoid inhibitor	CXCR4	NCT01837095	I	56	HER2-negative BC	Polyphor Ltd.	Balixafortide	Balixafortide/ eribulin	Total SAEs (38); febrile neutropenia (9), neutropenia (4), constipation (4), pneumonia (4), urinary tract infection (5)	• ORR 30% (CR 0%) • DCR 60%	N/A	Lancet Oncol 2018; 19(6):812-824
Non-lymphoid inhibitor	CXCR4	NCT01977677	I/II	29	GBM	Lawrence Recht, Professor of Neurology, Stanford University	Plerixafor (AMD3100)	Plerixafor/standard chemoradiation Standard chemoradiation	N/A	• Relative blood volume change at DSC-MRI 0.76 vs 0.88 at 1 month and 0.67 vs 0.87 ($p = 0.001$) at 6 mo • Out of field first recurrence rate 58.8% vs 10% ($p = 0.018$)	• Median OS 20.7 mo	J Clin Oncol 2018 36:15_suppl. 2019–2019

SCCHN, squamous cell carcinoma of the head and neck; IO, immunotherapy; NHL, non-Hodgkin lymphoma;CT, chemotherapy; NSCLC, non–small-cell lung cancer; DLT, dose-limiting toxicity; SLCL, small-cell lung cancer; MTD, maximum tolerated dose; BC, breast cancer; SAE, serious adverse event; TNBC, triple-negative breast cancer; BOR, best overall response; HR, hormone receptor; ORR, overall response rate; RCC, renal cell carcinoma; CR, complete response; HCC, hepatocellular carcinoma; PR, partial response; CRC, colorectal cancer; SD, stable disease; GBM, glioblastoma multiforme; DCR, disease control rate (CR + PR + SD); DLBCL, diffuse large B-cell lymphoma; OS, overall survival; CUP, carcinoma of unknown primary; PFS, progression-free survival; CRPC, castration resistance prostate cancer; iPFS, PFS as assessed by iRECIST; MSS, microsatellite stability; DFS, disease-free survival; MSI, microsatellite instability; w, weeks; mo, months; y, years; ICOS, inducible co-stimulator; GITR, glucocorticoid-induced TNFR-related; TNFR, tumour necrosis factor receptor; LAG3, lymphocyte activation gene-3; TLR, Toll-like receptor; IDO1, indoleamine-2,3-dioxygenase 1; TGFB, transforming growth factor beta; AST: Aspartate transaminase; CPK: Creatine phosphokinase; ALT: alanine aminotransferase, GMB: glioblastoma multiforme; HER: epidermal growth factor receptor 2 (*HER2*); DSC: differential scanning calorimetry; MRI: Magnetic Resonance Imaging.

polarisation towards effector lineages, in particular T-follicular helper cells, as its genetic deficiency impairs ability to mount appropriate germinal centre reaction and Ig class switch [37]. The earliest ICOS-engaging agent is the antibody JTX-2011, designed to achieve dual agonist/Treg-depleting activity, currently explored in the ICONIC trial alone or in combination with nivolumab [38]. GSK33359609, instead, is an IgG4 designed as a pure agonist, currently tested also in combination with pembrolizumab in the INDUCE-1 trial [39]. Other ICOS-targeting agents include MEDI-570, with stronger ADCC-mediated T cell–depleting activity that makes it unsuitable for cancer IO and is being tested in T-cell lymphomas, and KY1044 with dual agonist/depleting activity, still in preclinical research.

4.2. OX40

Key for CD4 memory establishment [40], OX40's role in Tregs is modulated by the cytokine environments [41]. Thus, the tumour microenvironment may strongly impact the pharmacodynamics of OX40-targeting agents. Preclinically, OX40 agonists are moderately effective alone and synergise with vaccination or immune modulators, especially anti-CTLA4 and 41BB but also surgery, chemotherapy and radiotherapy [42–44]. Relevant for trial design, sequential combination with anti-PD1 is additive, but simultaneous appears antagonistic [45,46].

OX40 modulators in development include monoclonal antibodies (MEDI6469, GSK3174998, INCAGN01949, MEDI0562, BMS-986178 and KY-B602) but also less conventional molecules like bispecific antibodies co-targeting PDL1 (MEDI1109) or CTLA4 (ATOR-1015), OX40L-Fc fusion proteins [47–50] and oncolytic viruses armed with OX40L (DNX-2440). The non-humanised IgG1 monoclonal 9B12 was the first agent tested in humans. Although data must be interpreted cautiously because of interspecies immunisation, its effect on memory T cells may suggest synergy with vaccination [51]. Globally, preliminary data with monoclonal antibodies show good safety as monotherapy and some activity [44]. Novel clinical settings and combinations are being explored: MEDI6469 in combination with radiotherapy in breast cancer (NCT01862900) and with radiotherapy + cyclophosphamide in prostate cancer (NCT01303705); MEDI0562 in the neoadjuvant setting of HNSCC and melanoma (NCT03336606). Recent preclinical data also support synergy with TLR agonists [52].

4.3. Glucocorticoid-induced TNFR-related

GITR is constitutively highly expressed in Treg and inducibly on NK and effector T cells, whereas its ligand (GITRL) is expressed on activated APCs and

endothelial cells [53,54]. Its role in the immune system is particularly complex and context dependent. GITR-null T lymphocytes show enhanced proliferation upon TCR engagement but also enhanced antigen-induced cell death [54,54]. Conversely, GITR engagement through ligands or agonistic antibodies promotes T effector cell expansion and cytokine production. On Tregs, GITR engagement may favour proliferation but also inhibit suppressive activity [54,55]. TRX518, an agylcosyl IgG1 with no expected Treg-depleting activity, was the first agent to initiate clinical development and showed unremarkable toxicity but little efficacy as monotherapy [56]. Preliminary results with other agents (BMS-986156, AMG228 and all IgG1) show a tolerable toxicity profile, characterised by a high incidence of fever (20–30%) [57,58]. Besides antibodies (INCAGN1876, GWN323, MK-4166 and MK-1248), agents in development include synthetic GITR ligands fused to an Fc: OMP-336B11, a GITRL trimer, and MEDI1873 [59], a GITRL hexamer. No clinical results are available.

4.4. 4-1BB

4-1BB provides CD28-independent costimulation able to overcome anergy [60]; agonists can eradicate established tumours even in monotherapy; combined with anti-PD1 they induce memory CD8-mediated long-term rejection of poorly immunogenic tumours [61,62]. Two agonistic antibodies have been developed to date. The IgG4 urelumab showed on-target dose-dependent and potentially fatal liver toxicity [63]; lowering doses in combination with nivolumab decreases efficacy [62]. Urelumab is now being explored with cetuximab and rituximab as an ADCC enhancer [64]. The IgG2 utomilumab, a weaker but safer agonist, showed preliminary efficacy in combination with pembrolizumab [65,66]. Novel strategies aim to enhance tumour specificity, through innovative scaffolds and drug targeting: bispecific molecules also binding tumour/stroma markers (PRS-343 targeting HER2 and PRS-343 targeting glypican 3), engineered with smaller antibody-mimetic scaffold (anticalin) and bicyclic peptides with potentially better tissue penetration than antibodies due to smaller size [67]. Promising results have been observed from preclinical studies, with an ameliorated toxicity profile in oncogenic mouse models [68].

5. Non-lymphoid inhibitors

Multiple non-lymphoid cells inhabit the complex tumour microenvironment and strongly influence the penetration, activation, differentiation and cytotoxic activity of antitumoural lymphoid cells [1,69]. Tumour-associated macrophages (TAMs) in particular correlate with poor survival [70]. Targets in this group include enzymes, growth factor receptors and pathogen recognition systems that can be engaged by chemically variegated agents.

5.1. Indoleamine-2,3-dioxygenase 1

IDO1 is a cytosolic haeme-containing enzyme catalysing the key step in the catabolism of tryptophan, first shown to be critical for maternal–foetal immune tolerance [71]. IDO1 inhibits immune activation through 3 main mechanisms: (i) mammalian target of rapamycin (mTOR) inhibition through tryptophan depletion [72–75]; (ii) formation of kynurenine, widely used as a pharmacodynamic biomarker, which promotes Tregs through the aryl hydrocarbon receptor [76] (iii) direct, non-enzymatic signalling through its ITIM domain (homologous to TIGIT) [77]. Tumours expressing IDO1 resist immune rejection, reversed by pharmacological IDO inhibition [78]. IDO1 is induced by interferon gamma and mediates primary or acquired resistance to anti-CTLA4 therapy [79,80]. There is debate as to whether the other tryptophan-metabolising enzyme Tryptophan 2,3-dioxygenase (TDO), predominantly expressed in the liver, plays any role in tumour immunity.

Owing to its enzymatic nature and well-characterised molecular structure, IDO1 is an ideal target for drug development and generated unprecedented interest, with several structurally unrelated and orally active agents identified to date, including natural compounds [3,81]. Initial promising results with indoximod, (D-methyl-tryptophan), further ignited interest [82]. Intriguing are the preliminary results (n = 46) of indoximod in combination with sipuleucel-T for patients with refractory metastatic prostate cancer, with an increase in PFS to 10.3 vs 4.1 months [83]. Combined with chemotherapy (gemcitabine + nab-paclitaxel), it showed preliminary activity in pancreatic adenocarcinoma (n = 30, ORR 37%) [84]. Indoximod is not a bona fide IDO1 inhibitor but more a tryptophan mimetic, directly relieving mTORC inhibition on lymphocytes [82,85]. Epacadostat is an IDO1-selective hydroxyamidine administered twice daily [86]. After phase 1 studies showing little toxicity at pharmacodynamically effective doses [87], large phase 2/3 trials were launched in combination with ICIs. However, the ECHO-301 failed to show superiority over pembrolizumab alone in melanoma [88]. This failure had a major impact on the entire field, causing a domino effect that halted or slowed down the development of all other IDO1-targeting compounds. Because of its complex biology, it is still unclear if IDO1 is an inherently ineffective target or if more potent and stable compounds like the fluoroquinolone derivative BMS-986205/F-001287 [89](administered once daily) may still have some room. NLG-919/GDC-0919/navoximod is an orally available IDO1 inhibitor with an imidazole backbone, with some activity also against TDO. In combination

with atezolizumab, preliminary results are slightly disappointing, with ORR not significantly different from what expected from atezolizumab monotherapy [90]. Numerous other compounds are in development (see Supplementary Table 1).

5.2. CSF-1 pathway

This pathway, key for monocyte-derived cells, includes CSF1R, a tyrosine kinase receptor of the plateled derived growth factor (PDGF) receptor family, and two structurally unrelated ligands: CSF1 (secreted in the bloodstream and acting at long and short ranges) and IL34 (undetectable in blood, acting at short range) [91]. Its blockade modulates the recruitment and phenotype of TAMs, significantly enhancing the efficacy of CTLA4, PD1/PDL1 or IDO1 inhibition [92,93]. T cells themselves can secrete CSF1 upon PD1 blockade, inducing secondary resistance [94]. Sensitivity to CSF1R inhibitors may be genetically influenced, especially in Southeast Asians [95].

CSF-1R pharmacological inhibition has been pursued by neutralising either antibodies or small kinase inhibitors [93]. Many agents are currently in development, making CSF1R pathway-targeting agents one of the largest classes of NGIM (Fig. 4) by molecules and patients. Major interest lies in Central Nervous System (CNS) and pancreatic tumours, where macrophages play dominant roles [96–98]. Emactuzumab has been extensively explored as targeted therapy in the CSF1R-expressing tenosynovial giant cell tumours, yielding ample information on its toxicity characterised by facial oedema and connective tissue autoimmunity [99]. It is currently tested with chemotherapy or IO (including CD40 inhibitors). Other antibodies inhibit binding of CSF1 (lacnotuzumab and PD-0360324) or both CSF1 and IL-34 (cabiralizumab and IMC-CS4).

Among tyrosine kinase inhibitors, BLZ945, a brain-penetrant and highly CSF1R-specific agent, has been most extensively characterised preclinically in studies with a transgenic mouse glioma model, which revealed the frequent development of IGF1-dependent secondary resistance in long-term treated mice [98]. BLZ945 is being tested in phase 1/2 with anti-PD1 PDR001 [100]. Many other less CSF1R-specific kinase inhibitors are in preclinical or early clinical phase of development: pexidartinib/PLX3397 (the most advanced), linifanib/ABT-869 (an ATP competitive primarily active against Vascular endothelial growth factor receptor (VEGFR)/Plateled derived growth factor receptor (PDGFR)); OSI-930, GW2580 and ARRY-382.

5.3. Transforming growth factor beta

TGFb acts through multiple canonical (SMAD-mediated) and non-canonical signalling pathways and has pleiotropic effects, promoting metastatisation of cancer cells but also polarising immune cells to immunosuppressive phenotypes and inhibiting NK cytokine production [101]. TGFb is produced mostly by stromal cells and participates in tumour-associated connective tissue remodelling, in particular, radiation-induced fibrosis which interferes with radio-induced T cell priming to tumour antigens [102], perhaps modulating abscopal effects. Preclinical studies with TGFb-targeting agents have yielded somewhat contradictory results [101]. TGFb targeting has been attempted through multiple avenues that include antisense oligonucleotides, neutralising antibodies to the receptor or the ligand, and TGFb receptor kinase inhibitors (ALK5) [101]. Kinase inhibitors are likely to have little effect on non-canonical pathways, possibly resulting in a different toxicity spectrum including off-target effects on pathways triggered by TGFb-like ligands (Activin or Nodal) [103]. The ALK5 inhibitor galunisertib and pharmacologically similar compounds show cardiac toxicity, possibly overcome by optimising the schedule [104]. An intriguing strategy to minimise systemic toxicity and increase activity is dual TGFb/PD1 inhibition, as in M7824, a bifunctional molecule coupling the anti-PDL1 antibody avelumab with a TGFb 'trap', consisting of the soluble fraction of the TGFbR2 [105]. Results are promising (for instance in Human Papilloma Virus (HPV) + tumours) but with non-negligible toxicity.

5.4. CD47-SIRPa

This axis represents a crucial 'don't eat me signal' essential for self recognition, in particular to avoid elimination of circulating haematopoietic stem cells. CD47 is a transmembrane protein widely expressed in normal and tumoural cells; its receptor SIRPα is another ITIM-containing signalling protein, expressed on myeloid cells, especially macrophages. CD47 ablation stimulates macrophage phagocytosis and polarisation and synergises with PD1 blockade. The axis has been targeted pharmacologically mostly by antibodies against CD47, but also recombinant polypeptides and bispecific molecules [106]. The recent discovery that the SLAMF7 receptor is crucial for CD47-mediated phagocytosis [107] may provide a biomarker to identify putative responders. No mature clinical data are available, but the field has generated significant interest to date [108].

5.5. Chemokines

Chemokines are low-weight and short-range–acting proteins which mediate angiogenesis, metastasis and recruitment/polarisation of immune cells through their action on G-protein receptors. Central for their

pharmacology is their high ligand promiscuity and receptor redundancy, which may suggest the design of multifunctional inhibitors [109]. Of the 20 receptors and 47 ligands known, the CXCL12(SDF1)/CXCR4 axis has received most attention in oncology.

CXCR4 receptor mediates not only survival and metastasization in tumours but also homing of haematopoietic stem cells. Plerixafor, approved for HSC mobilisation in autologous transplant, is the most studied agent. CXCR4 inhibition could revert tolerogenic phenotype of infiltrating myeloid and Treg cells, synergising with PD1/CTLA4 blockade [110,111]. Preliminary activity has been observed in gastrointestinal and CNS tumour. Although promising, in solid tumours, the strategy of combining CXCR4 inhibitors and anti-PD(L)1 agents has disappointed, with at least two studies with the fully human IgG4 ulocuplumab terminated for the lack of efficacy. Several other agents belonging to multiple chemical classes are in early development [112]. The inhibiting peptide balixafortide received Food and Drug Administration (FDA) fast track designation in combination with eribulin for breast cancer [113]. The pegylated RNA oligonucleotide NOX-A12/olaptesed pegol, neutralising the ligand CXCL12, received orphan drug designation from the FDA for treatment of glioblastoma in association with radiotherapy and is being investigated with pembrolizumab. Many of these agents have shown promising results in haematological malignances (not discussed here) and are moving to solid tumours, like the CCR4 inhibitor mogamulizumab with anti-PD1.

6. Non-lymphoid stimulators

6.1. CD40

CD40, a TNFR family member, 'licenses' APCs to activate cytotoxic CD8 and NK cells, bypassing CD4 helpers and upstream innate pathways. CD40-dependent antitumoural response can be enhanced by chemotherapy in immunologically 'cold' tumours like pancreatic cancer, possibly through stroma remodelling. CD40 agonists, including antibodies but also recombinant ligands, have been developed for about 20 years, with encouraging results especially in haematological malignances but also early signals in solid tumours. As expected, toxicity is dominated by immune hyperactivation with cytokine release syndrome, thromboembolic events and liver enzyme alteration, although manageable and rarely high grade [114]. Unexpectedly, engagement of inhibitory Fc receptor enhances CD40 agonism [115], prompting the design of IgG1 antibodies. A recent study systematically analysed the structure–function relationship of most existing anti-CD40 antibodies [116]. Preclinical studies support synergism with CSF1R blockade [117].

6.2. PAMP and DAMP receptors (Toll-like receptor, RIG-I–like receptor and cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes)

Pathogen-associated molecular patterns and damage-associated molecular patterns receptors (PAMP/DAMP-R) recognise chemically heterogeneous molecules shared by pathogenic organisms, either intracellular (e.g. unmethylated CpG-rich DNA from viruses or senescent or radiation-killed human cells) or extracellular (e.g. bacterial lipids and lipopolysaccharide, flagellin). They include TLRs, RIG-I–like receptors and sensors of cytosolic cyclic nucleic acids acting through the stimulator of interferon genes. These receptors are variably expressed by multiple cell types, including lymphocytes, but TLR7/8/9, the most relevant in the setting of metastatic cancer, are predominantly expressed and functional on monocytes and dendritic cells, where they induce the production of interferon and other cytokines and enhance antigen presentation, possibly converting immunologically 'cold' tumours into 'hot' [118]. Many TLR agonists, approved (attenuated Bacillus Calmette–Guérin (BCG), monophosphoryl lipid A and imiquimod) or in development (Hiltonol), are employed either as vaccine adjuvants or as topical agents [119]. Systemically administered TLR7/8 and 9 agonists in combination with chemotherapy were widely investigated in late 2000s but intolerable toxicity hampered further development [120]. More recently, the observation of abscopal effect after local therapy provided momentum for testing intratumoral administration, which combined with systemic ICIs may achieve long-range efficacy with localised toxicity. The TLR9 agonist CMP-001, a CpG oligonucleotide, recently showed intriguing results in combination with pembrolizumab in pretreated melanomas. The TLR8 agonist motolimod, however, failed to improve survival when added to standard chemotherapy in ovarian and head and neck cancers [121]. This is a (re) growing field that is generating a large interest. However, clinical experimentation with intratumoural agents has some methodological peculiarities; recently, a panel of experts gathered to issue guidelines for adequate trial conduct and reporting [122].

7. Agents targeting NK cells

NK cells sit at the intersection between the adaptive and the immune response and play a crucial role in tumour immunity [123,124]. Being antigen independent, NK cells can theoretically be effective in poorly immunogenic tumours. Major NK-specific targets include the inhibitory receptors, killer-cell immunoglobulin-like receptors (KIRs) and CD94/NKG2A, and the activating receptors, NKG2D, NKp30, 2B4, DNAM1 and IL15 [124]. NK cells can contribute to antitumoural immune response by either ADCC or antibody-independent cytotoxicity. Thus, NK enhancement is being pursued

either in combination with antibodies targeting oncogenic drivers (e.g. cetuximab) or with anti-PD(L)1 agents. Agents in clinical development include IPH2102/lirilumab (a KIR2D antagonist) and IPH2201/monalizumab (an NKG2A antagonist) [122]. The toxicity profile of these agents is very favourable [125]. Recently, ALT-803, a complex IL-15 superagonist, showed promising results in combination with nivolumab in IO-pretreated NSCLC [126]. Several trials have been initiated with this compound.

8. Conclusions

The landscape depicted previously has generated exceptionally high expectations in all stakeholders (patients, doctors and pharma), exposing the field to 'bubbles' like the ongoing IDO1 story, which prevent rational resource allocation and adequate data interpretation. Some target pathways are particularly overcrowded with several drugs competing for the same therapeutic space. These agents have certainly great potential of enhancing the benefit of IO, possibly allowing the 'conquest of unredeemed land' represented by immunologically cold diseases. However, major challenges should be overcome. First, the availability of adequate predictive and pharmacodynamic biomarkers. Molecular characterisation through next-generation sequencing, which has accelerated the development of targeted drugs, currently appears of little use in immuno-oncology, besides the quantification of mutational burden, not currently known to be predictive of response to non-PD1 agents. Immunohistochemical or RNA-based characterisation of the infiltrate may be necessary, something that cannot be achieved through liquid biopsy. Secondary to the biomarker issue is appropriate trial design. Proper evaluation of therapeutic benefit by immunotherapeutic agents requires quantification of durable survival over extended observation time [127]. Conventional 'A vs B' phase 3 trial design is unlikely to provide definitive answers on efficacy within reasonable time and cost. 'Platform' trials with adaptive designs, pioneered in the field of targeted therapy, may optimise resources but require considerable effort and collaboration between pharma and academia. Some notable examples are already underway, like the FRACTION, ADVISE, JAVELIN and QUILT platforms.

Conflict of interest statement

G.C. received personal fees from Seattle Genetics, Pfizer, Lilly, Novartis, Roche and Ellipsis for consultation. Other authors have no conflict of interest.

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Appendix A. Supplementary data

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