Targeting Cbl-b in cancer immunotherapy

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ABSTRACT

Cancer immunotherapy with immune-checkpoint blockade has improved the outcomes of patients with various malignancies, yet a majority do not benefit or develop resistance. To address this unmet need, efforts across the field are targeting additional coinhibitory receptors, costimulatory proteins, and intracellular mediators that could prevent or bypass anti-PD1 resistance mechanisms. The CD28 costimulatory pathway is necessary for antigen-specific T cell activation, though prior CD28 agonists did not translate successfully to clinic due to toxicity. Casitas B lymphoma-b (Cbl-b) is a downstream, master regulator of both CD28 and CTLA-4 signaling. This E3 ubiquitin ligase regulates both innate and adaptive immune cells, ultimately promoting an immunosuppressive tumor microenvironment (TME) in the absence of CD28 costimulation. Recent advances in pharmaceutical screening and computational biology have enabled the development of novel platforms to target this once 'undruggable' protein. These platforms include DNA encoded library screening, allosteric drug targeting, small-interfering RNA inhibition, CRISPR genome editing, and adoptive cell therapy. Both genetic knock-out models and Cbl-b inhibitors have been shown to reverse immunosuppression in the TME, stimulate cytotoxic T cell activity, and promote tumor regression, findings augmented with PD1 blockade in experimental models. In translating Cbl-b inhibitors to clinic, we propose specific gene expression profiles that may identify patient populations most likely to benefit. Overall, novel Cbl-b inhibitors provide antigen-specific immune stimulation and are a promising therapeutic tool in the field of immuno-oncology.

BACKGROUND

The advent of immune checkpoint inhibitors (ICI), specifically cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death (ligand) protein 1 (PD1/PD-L1) blockade, brought immunotherapy into standard treatment paradigms for patients across many advanced cancers. However, the clinical expansion of these agents is plateauing with beneficial outcomes seen in only a minority of patients.[1] While efforts are underway to identify more predictive biomarkers of current ICI, the investigation of novel cellular and molecular targets will be necessary to drive more effective and sustained antitumor immunity, reverse immunosuppression in the tumor microenvironment (TME), and further advance immune-based therapies.[2]

The PD1 and CTLA-4 checkpoints represent only two of many transmembrane receptor-ligand pairs dictating T cell antigenic response. A host of costimulatory and coinhibitory molecular interactions have been characterized between T cells, antigen presenting cells (APCs), and tumor cells.[3] While antibody targets have now been developed against some of these interactions, pharmacological intervention against the downstream signaling pathways has been limited.[4] These intracellular pathways, ultimately responsible for the transcriptional and post-transcriptional regulation of T cell activation, can serve as resistance and bypass mechanisms against extracellular checkpoint therapy.[5 6] Additionally, a paucity of costimulatory receptors or neoantigenicity in the TME of some tumors may limit the use of ICI and therapies targeting these cell-surface interactions altogether.

Success in the next phase of immuno-oncology (IO) will depend on addressing unmet needs and leveraging lessons learned from prior clinical trials.[7] Thus future and ongoing goals include: (1) developing biomarker-driven models to predict immunotherapy response based on tumor molecular and genomic characteristics; (2) focusing on the tumor biology of non-responding patients to identify resistance pathways; (3) identifying all potential avenues of immunosuppression in the TME (eg, metabolic insufficiency, hypoxia, adenosine signaling, regulatory cells); (4) facilitating sufficient levels of infiltrating and durably activated T cells; and (5) applying advanced pharmacologic techniques against previously inaccessible proteins and pathways. By addressing this needs assessment, future clinical trials may more effectively combine standard of care ICI with novel immune-based therapies and targeted agents.

Casitas B lymphoma-b (Cbl-b) is an E3 ubiquitin ligase initially characterized as a

proto-oncogene but now understood to have a central role in regulating effector T cell function.[8] Cbl-b has been identified as a key inhibitor of T cell activation in the absence of CD28 costimulation.[9] Through a complex interaction of signal transducers, Cbl-b inhibits T cell transcriptional activity and promotes immune tolerance across innate and adaptive immunity.[6] As an intracellular master regulator, Cbl-b inhibition may represent a more specific and efficient route toward broad immune activation regardless of upstream checkpoint signaling (ie, CD28, CTLA-4). Importantly, recent advances in immunology, pharmacology, and data science have enabled the development of novel agents against intracellular proteins, such as Cbl-b.

Here, we will focus on the CD28 pathway, the central role of Cbl-b, and the impact on immune cell subsets by this ubiquitin ligase. We will discuss the preclinical development of Cbl-b inhibition, ranging from small-interfering RNA (siRNA) to allosteric regulatory targets to adoptive T cell therapy. Finally, upcoming clinical trials, combinatorial strategies, relevant biomarkers, and potential toxicities of Cbl-b inhibition will be considered.

T cell activation and regulation: an overview of costimulatory and coinhibitory receptors

Characteristics of the TME associated with an optimal cytotoxic antitumor immune responses include an inflammatory cytokine profile, favorable metabolic milieu, decreased regulatory cell activity, and T cell invasion and activation.[10] Dogmatically, CD8$^+$ T cell activation first requires antigen presentation via major histocompatibility complex II (MHC-II) on APCs. Without simultaneous costimulation, or in the presence of coinhibitory competition, however, T cells enter an anergic or apoptotic state. A diverse set of costimulatory and coinhibitory receptors have been characterized that influence this process.[5] While evolutionarily conserved to limit T cell overactivation, tumors can evade systemic immunity by 'hijacking' this delicate balance of checkpoints in favor of inhibitory pathways.[11]

Costimulatory receptors

The CD28 costimulatory receptor is an Ig family receptor that plays a central role in canonical T cell activation. Constitutively active in a majority of CD4$^+$ and CD8$^+$ T cells, CD28 binds to the CD80 (B7-1) and CD86 (B7-2) ligands expressed on APCs, ultimately promoting a T cell effector response.[12] CD80 and CD86 also compete for the CD28 homolog receptor, CTLA-4, expressed on the surface of T cells.[13] CTLA-4 binds these ligands with higher affinity as compared with CD28, maintaining a homeostatic immune response to repeated antigenic presentation. Sequential and differential ligation of CD86 with CD28 is followed by CD80 with CTLA-4—this process initiates cytotoxicity but prevents an unchecked immune response.[14] On stimulation, the cytoplasmic domain of CD28 initiates a phosphorylation cascade via PI3K and ZAP70, resulting in proteasomal degradation

of Cbl-b, a key inhibitory enzyme, and activation of the NFAT and NF-kB transcription factors (figure 1A).[6 15] This multifaceted cascade, activated via TCR/CD3 and CD28 stimulation, ultimately promotes transcriptional upregulation of IL-2, cytoskeletal recruitment, and overall cellular proliferation.[16] Unfortunately, early trials with the CD28 'superagonist' TGN1412 lead to severe cytokine release syndrome (CRS), likely due to the constitutive expression of this coreceptor along with the lack of TCR/CD3 primary stimulation.[17] These results led to an extended pause in cancer clinical trials addressing the CD28 pathway.

Given the initial clinical complexity of targeting CD28, a number of costimulatory receptors became high priorities as potential cancer immunotherapy targets, including 4-1BB, OX40, GITR, ICOS, CD27, and CD40 (table 1). In vivo experiments with 4-1BB agonism showed a dramatic increase in CD8$^+$ T cell proliferation, yet overall reduction in B, NK, and CD4$^+$ T cell activity.[18] This dichotomous effect, at least in part due to indoleamine 2,3-dioxygenase (IDO) signaling, may partially explain the poor humoral and innate immune response seen with 4-1BB stimulation. A phase I clinical trial with a 4-1BB agonist (urelumab) demonstrated preliminary antitumor activity in advanced cancers,[19] although further translation was hampered by hepatic toxicity and lack of target population with clear benefit.[20] Multiple next generation approaches targeting 4-1BB are in clinical development, taking advantage of affinity engineered multispecific antibodies (eg, GEN1046, NM21-1480, DSP107).[21 22] OX40, part of the TNF receptor family, is a costimulatory molecule with only transient expression in activated T cells.[5] Unfortunately, phase I/II trials with the OX40 agonists MEDI0562, BMS-986178+/-checkpoint blockade, and BGB-A445+/-tislelizumab failed to demonstrate clinical efficacy.[23 24] This lack of effect could be explained by a complicated kinetic relationship whereby receptor occupancy leads to receptor downregulation.[25] Similar to OX40, GITR ligation leads to NF-kB transcriptional activity and an overall decreased threshold of CD8$^+$ T cell activation.[26] The safety and efficacy of a GITR agonist, INCAGN-1876, is currently being studied alongside IDO and PD1 blockade in advanced cancers.[27 28] Additional GITR agonists under investigation include GWN323+/-spartalizumab, TRX518+/-chemotherapy or PD1 blockade, and BMS-986156+/-nivolumab; though phase I trials have shown minimal single-agent or combined efficacy.[29 30] The ICOS costimulatory molecule is also induced on primary T cell activation,[31] however, an early phase trial of the ICOS agonist, JTX-2011, alone or in combination with anti-PD1 was discontinued in 2020.[32] An additional agonist, feladilimab, has also shown disappointing results in phase II clinical trials alongside anti-PD1 therapy.[33] In contrast to most other costimulatory receptors, CD27 is constitutively active in naïve and regulatory T cells but poorly expressed on differentiation.[34] This pattern is advantageous for low affinity TCRs and stimulates effector function even in the absence of a strong antigenic presentation. Preliminary results from
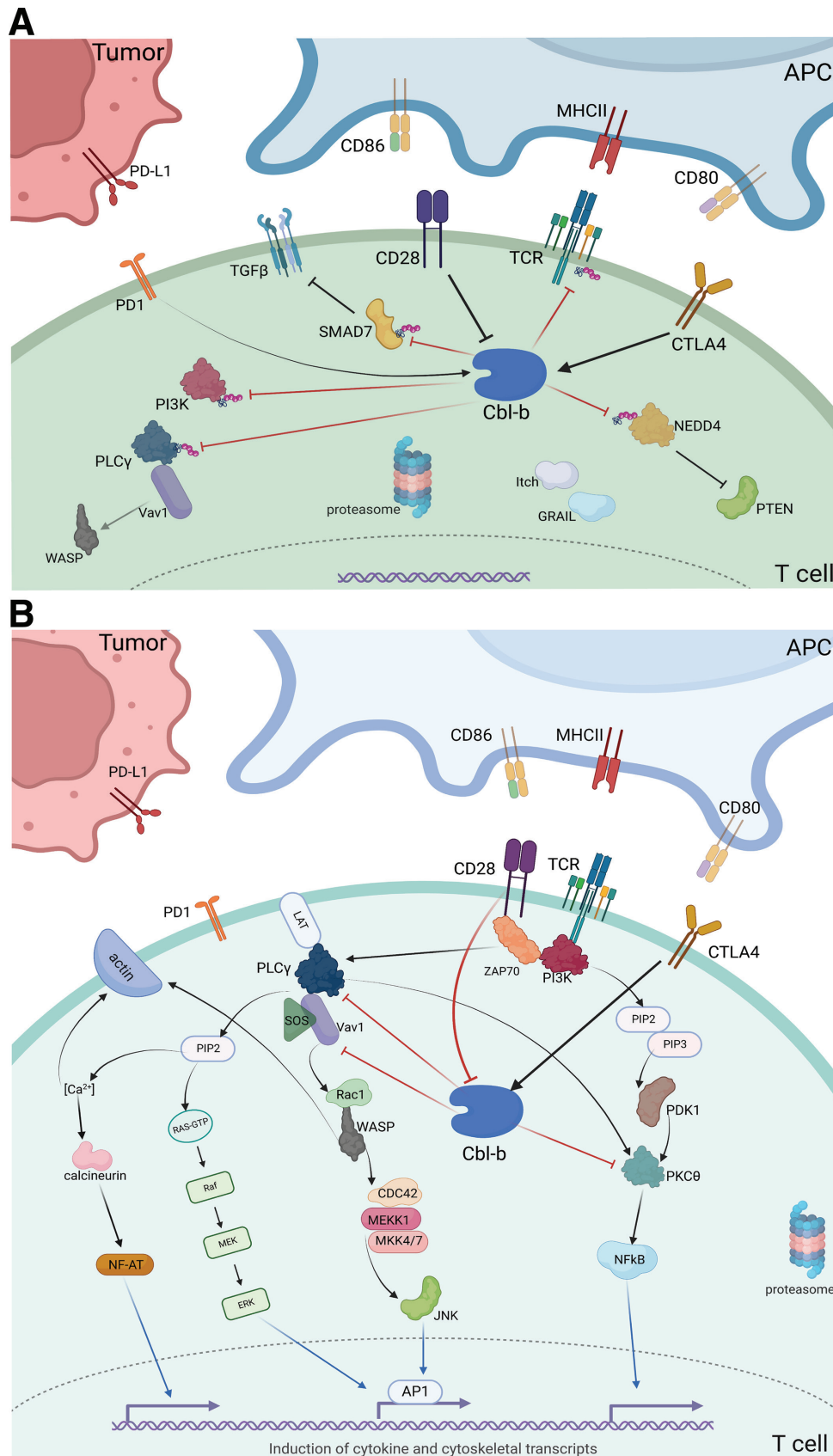
Figure 1 (A) T cell stimulation via CD28 costimulatory signaling cascade. (B) Depiction of intracellular Cbl-b signaling in the tumor microenvironment. CD28 costimulation inhibits downstream Cbl-b signaling, while CTLA4 stimulation promotes Cbl-b activity. On activation, Cbl-b ubiquinates several key proteins that inhibit effector function, promoting an immunosuppressive phenotype. *Red blocked arrows indicate direct ubiquitination. APC, antigen presenting cell; Cbl-b, Casitas B lymphoma-b; MHCII, major histocompatibility complex II.

Table 1 A non-exhaustive list of agents targeting costimulatory receptors with associated clinical trials in the field of immuno-oncology

Agents targeting costimulatory receptors

Costimulatory receptor	Receptor class	Agent, mechanism of action	Pharmaceutical	Trial	Outcomes
CD28	Ig	TGN1412 *CD28 "superagonist"*	TeGenero	Phase I NHS (2006)	Cytokine storm in initial six patients
		TAB08, theralizumab (rebranded TGN1412)	TheraMAB	Phase I, advanced tumors, NCT03006029	NA
		REGN5678 PSMA x CD28	Regeneron	Phase I/II, mCRPC, plus cemiplimab, NCT03972657	Dose-dependent reduction in PSA with moderate antitumor activity in higher-dose cohorts
		FPT155 CD80 Fc fusion protein, blocks CTLA-4 from competing for endogenous CD80	Five Prime, Amgen	Phase I, advanced tumors,±pembrolizumab, NCT04074759	NA
		ALPN-202, davoceticept, CD80 vIgD-Fc fusion protein & dual PD-1/CTLA-4 inhibitor	Alpine	Phase I (NEON-1), advanced tumors NCT04186637	2/48 PR, 23/48 SD
				Phase I (NEON-2), plus pembrolizumab, NCT04920383	Terminated (two grade 5 AEs)
4-1BB (CD137)	TNF	BMS-663513, urelumab, agonist mAb	BMS	Phase I, advanced tumors (NCT00309023), and melanoma (NCT00612664)	Enrollment paused at higher doses due to severe hepatotoxicity; lower doses tolerable but efficacy similar to anti-PD1
				Phase I, advanced CRC or H&N, plus cetuximab, NCT02110082	Only biomarker analysis reported
				Phase I/II (INTRUST), advanced tumors, intratumoral urelumab, plus nivolumab, NCT03792724	NA
				Phase I, PDAC, neoadjuvant/adjuvant GVAX, nivolumab, urelumab, NCT02451982	Non-significant improvement in DFS with urelumab addition
		PF-05082566, utomilumab, agonist mAb	Pfizer	Phase I, advanced tumors, NCT01307267	2/55 CR/PR
				Phase I, advanced CRC, plus irinotecan, cetuximab, NCT03290937	NA
				Phase I, advanced tumors, plus anti-CCR4, NCT02444793	Terminated
				Phase I, ovarian cancer, plus TIL therapy+aldesleukin, NCT03318900	NA
				Phase II, HPV+HNSCC, plus ISA101b vaccine, NCT03258008	NA

Table 1 Continued

Agents targeting costimulatory receptors

Costimulatory receptor	Receptor class	Agent, mechanism of action	Pharmaceutical	Trial	Outcomes
		GEN1046 4-1BB x PD-L1	Genmab, BioNTech	Phase I, advanced tumors, NCT03917381	40/61 PR or SD
				Phase II, advanced NSCLC,±pembrolizumab, NCT05117242	NA
		NM21-1480 4-1BB x PD-L1 x HSA	Numab	Phase I/II, advanced tumors, NCT04442126	NA
		DSP107 4-1BB x SIRPα	KAHR	Phase I, advanced tumors,±atezolizumab, NCT04440735	11/22 SD in dose-escalation phase
OX40	TNF	MEDI0562 agonist mAb	MedImmune, AstraZeneca	Phase I, advanced tumors, NCT02318394	2/55 PR, 24/55 SD
				Phase I, advanced tumors, plus durvalumab or tremelimumab, NCT02705482	Minimal single-agent or combination benefit
				Phase I, neoadjuvant HNSCC or melanoma, NCT03336606	NA
		BMS-986178 agonist mAb	BMS	Phase I/II, advanced tumors,±nivolumab and/or ipilimumab, NCT02737475	No objective response in monotherapy; up to 13% response in combination cohorts
				Phase I, advanced tumors, plus TLR9-agonist, NCT03831295	NA
		BGB-A445 agonist mAb	BeiGene	Phase I/II, advanced tumors,±tislelizumab, NCT04215978	NA
		MOXR0916 agonist mAb	Genentech	Phase I, advanced tumors, NCT02219724	2/173 PR, 57/173 SD
				Phase I, advanced tumors, plus atezolizumab, NCT02410512	11/70 SD
		PF-04518600, ivuxolimab, agonist mAb	Pfizer	Phase I/II, advanced tumors, avelumab±ivuxolimab +/- utomilumab±radiation NCT03217747	NA
				Phase I, advanced tumors±utomilumab, NCT02315066	14/57 SD

Continued

Table 1 Continued

Agents targeting costimulatory receptors

Costimulatory receptor	Receptor class	Agent, mechanism of action	Pharmaceutical	Trial	Outcomes
ICOS	Ig	JTX-2011, vopratelimab, agonist mAb	Jounce	Phase I/II, advanced tumors,±nivolumab, pembrolizumab, or ipilimumab, ICONIC, NCT02904226	Preliminary antitumor activity with nivolumab
				Phase II (EMERGE), NSCLC or urothelial cancer, plus ipilimumab, NCT03989362	Minimal benefit
				Phase II, TIS^{vopra}+NSCLC, anti-PD1±JTX-2011, SELECT, NCT04549025	Minimal benefit versus anti-PD1 alone
		GSK3359609, feladilimab, agonist mAb	GSK	Phase II (INDUCE-3), PD-L1 positive HNSCC, plus pembrolizumab, NCT04128696	Terminated
				Phase II/III (INDUCE-4), recurrent/metastatic HNSCC, GSK3359609 versus placebo plus pembrolizumab & chemo, NCT04428333	Terminated
				Phase I/II, advanced tumors, plus trememlimumab, NCT03693612	NA
GITR	TNF	INCAGN-1876 agonist mAb	Incyte, Agenus	Phase I/II, advanced tumors, NCT02697591	NA
				Phase I/II, advanced tumors,±nivolumab/ ipilimumab, NCT03126110	NA
				Phase II, HNSCC, plus retifanlimab (PD1), NCT05359692	NA
				Phase II, GBM, plus retifanlimab (PD1), NCT04225039	NA
		BMS-986156 agonist mAb	BMS	Phase I/II, advanced tumors,±nivolumab, NCT02598960	Preliminary antitumor activity in combination cohort
				Phase I/II, advanced lung or liver, ipilimumab, nivolumab±radiation, NCT04021043	NA
		GWN323 agonist mAb	Novartis	Phase I, advanced tumors,±spartalizumab, NCT02740270	Minimal single-agent benefit; 18/45 with disease control in combination cohort
		TRX518 agonist mAb	Leap	Phase I, advanced tumors, NCT01239134	Minimal single-agent benefit
				Phase I, advanced tumors, plus gemcitabine, pembrolizumab, or nivolumab NCT02628574	Minimal monotherapy or combination benefit

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Table 1 Continued

Agents targeting costimulatory receptors

Costimulatory receptor	Receptor class	Agent, mechanism of action	Pharmaceutical	Trial	Outcomes
CD27	TNF	CDX-1127, varlilumab, agonist mAb	Celldex	Phase I, advanced tumors, NCT01460134 (CDX)	1/31 PR; 8/31 SD in advanced solid tumors
				Phase I/II, advanced tumors, plus nivolumab, NCT02335918	Response similar to nivolumab monotherapy
				Phase I, advanced tumors, plus atezolizumab, NCT02543645	Terminated
				Phase I/II, stage IIB-IV melanoma, 6MHP vaccination±CDX-1127, NCT03617328	NA
		MK-5890 agonist mAb	Merck	Phase I, advanced tumors,±pembrolizumab, NCT03396445	4/14 CR/PR in combination cohort
				Phase II, advanced NSCLC, KEYMAKER-U01C substudy, NCT04165096	NA
CD40	TNF	RO7009789, selicrelumab, agonist mAb	Hoffmann-La Roche	Phase I, advanced tumors, plus atezolizumab, NCT02304393	No improvement compared with anti-PD1 monotherapy
				Phase I, advanced tumors, plus anti-CSF1R, NCT02760797	No objective clinical benefit
				Phase I, advanced tumors, plus anti-VEGF, NCT02665416	NA
		CDX-1140 agonist mAb	Celldex	Phase I, advanced tumors,±Flt3L, pembrolizumab, or chemotherapy, NCT03329950	NA
				Phase I, IB-IV melanoma (including uveal), plus neoantigen vaccines and TLR-agonist NCT04364230	NA
				Phase I, TNBC, plus Flt3L, chemo, NCT05029999	NA
				Phase I, advanced NSCLC, plus Flt3L, radiation, NCT04491084	NA
				Phase I, advanced epithelial cancers, TSA-reactive TCR-engineered T cells, pembrolizumab, NCT04520711	NA

Table 1 Continued

Agents targeting costimulatory receptors

Costimulatory receptor	Receptor class	Agent, mechanism of action	Pharmaceutical	Trial	Outcomes
		APX005M, sotigalimab, agonist mAb	Apexigen	Phase I/I, advanced NSCLC or melanoma, plus nivolumab, NCT03123783	6/33 PR, 3/33 SD
				Phase I, untreated PDAC, plus chemo±nivolumab, NCT02482168	14/30 PR, 8/30 SD
				Phase II, untreated PDAC, plus chemo±nivolumab, NCT03214250	1-year OS 57.3% (APX005M+chemo+nivolumab), p=0.007, n=34
				Phase II, resectable neoadjuvant esophageal adenocarcinoma, plus chemo, radiation, NCT03165994	NA
				Phase I, advanced melanoma or RCC, plus nivolumab/ipilimumab, NCT04495257	NA
		ChiLob7/4 agonist mAb	University of Southampton, UK	Phase I, advanced tumors, NCT01561911	SD in 15/29 treatments
		SEA-CD40 agonist mAb	Seagen	Phase I, advanced tumors,±pembrolizumab, or±pembrolizumab and chemo, NCT02376699	NA

AE, adverse event; CR, complete response; DFS, disease-free survival; mAb, monoclonal antibody; OS, overall survival; PR, partial response; SD, stable disease; TNF, tumor necrosis factor; TSA, tumor-specific antigen.

a phase II trial assessing the CD27 agonist, varlilumab, alongside PD1 blockade showed only marginal response rates in patients with ovarian and colorectal cancer.[35]

While initially promising, these costimulatory agonists have yet to demonstrate meaningful benefit in clinical trials. Potential explanations include the dichotomous effects on various immune cell subsets, transient expression of the target receptors, and lack of biomarkers identifying patient populations most likely to benefit. In contrast, the CD28 pathway is constitutively expressed with direct links to the clinically validated CTLA-4 axis.[12 14] Furthermore, CD28 signaling is required for response to PD1 therapy.[36] Thus, renewed efforts have emerged to safely and more effectively stimulate this coreceptor.

For example, bispecific antibodies have been developed to simultaneously target a tumor-specific antigen (TSA) and CD28 for epithelial tumors, with a PSMAxCD28 agent showing preliminary antitumor activity alongside anti-PD1 (cemiplimab) in a phase I/II trial of patients with metastatic prostate cancer (table 1).[37 38] Building off bispecific TSAxCD3 antibodies that initiate primary TCR stimulation,[39] the TSAxCD28 platform provides costimulation while maintaining crucial specificity. Despite severe side effects from the original CD28 'superagonist', TGN1412 was rebranded as TAB08 (TheraMAB) and has cautiously re-entered clinical development.[40] Additionally, Fc fusion proteins of CD80 (an innate CD28 and CTLA-4 ligand) have also been developed and shown increased T cell effector response in preclinical studies.[41] The CD80-Fc fusion protein, FPT155, has shown no evidence of CRS (indicating the requirement of antigenic TCR stimulation), higher affinity for CD28 over CTLA-4, and augmented efficacy alongside anti-PD1 therapy in a CT26 murine model.[42] Further, the NEON-1 trial assessing ALPN-202, a PD-L1 dependent CD28 costimulator and CTLA-4 inhibitor, has shown clinical benefit in 25 of 48 patients, though augmentation with pembrolizumab exhibited high-grade AEs (NEON-2).[43 44] Finally, given the toxicity of TGN1412, marginal efficacy of costimulatory receptor agonists, and the potential to overcome anti-PD1 resistance, attention has turned to downstream regulators of the CD28 axis (eg, Cbl-b) despite the challenges in targeting these intracellular proteins.[17]

Coinhibitory receptors: CTLA-4 & PD-1

Antibodies against the CTLA-4 and PD-1 coinhibitory receptors have set a new benchmark for clinical benefit. However, many common malignancies (eg, ovarian, colon, prostate) do not respond and, for relatively responsive cancers (eg, melanoma, non-small cell lung cancer (NSCLC)), a majority of patients develop resistance.[45] As mentioned alongside its Ig-receptor homolog, CD28, CTLA-4 exhibits strong affinity to the CD80/CD86 ligands on APCs.[46] Downstream signaling ultimately negates actin recruitment, transcription factor activity, and effector response.[46] This inhibitory action is also regulated via Cbl-b, the expression of which is significantly elevated on CTLA-4 ligation.[16] Unlike CD28, CTLA-4 is

not constitutively expressed[5]; translocation only occurs after TCR stimulation, making it a slightly more focused pharmaceutical target in the TME.

PD1, perhaps the most well-recognized co-inhibitory receptor, is another homolog to CD28. Ligation with PD-L1 leads to inhibition of CD28-mediated PI3K activity, promoting T cell anergy.[47] Clinical trials have demonstrated superior outcomes with anti-PD1 versus anti-CTLA-4 therapy in many cancers, though precise mechanisms behind these differential outcomes are less clear. While gene expression analyses have identified certain genes (eg, Bcl-xL, JAK2) as potential mediators of both anti-PD1 efficacy and resistance,[48] functional assays in Cbl-b knockout (KO) mice showed a complete lack of tumor regression with PD-L1 blockade as compared with wild-type controls. These results in syngeneic melanoma murine models highlight Cbl-b as a critical, downstream mediator of PD(L)1 signaling, a finding validated by multiple groups.[49] Additionally, PD-L1 silencing in murine dendritic cells (DCs) was shown to reduce Cbl-b expression in CD8^{+} T cells,[50] providing additional data linking these proteins to similar immune pathways. Overall, Cbl-b not only mediates CTLA-4 signaling but also PD1-induced immunosuppression in the TME.

Intracellular signaling: the central role of Cbl-b, an E3 ubiquitin ligase

Despite the promise of coinhibitory receptor blockade (ie, ICI), the most potent antitumor immune responses may yet be harnessed via direct augmentation of T cell activation machinery. The costimulatory receptor, CD28, provides a critical secondary signal to promote T cell activation and prevent anergy on TCR/MHC-II ligation. Following T cell activation, CTLA-4 expression escalates to outcompete the binding of CD28 to its APC ligands (CD80, CD86),[4] reducing the risk of unnecessary T cell proliferation under physiologic conditions. Cbl-b, a RING finger E3 ubiquitin ligase, is a key intracellular mediator between CD28 and CTLA-4 receptor signaling (figure 1B). Without CD28 stimulation, Cbl-b, along with the ligases Itch and GRAIL, lead to ubiquitination of several key proteins—namely, PI3K and PLCγ, the zeta-subunit of TCR, and NEDD4.[15 16] Ultimately, this ubiquitination prevents Vav1-mediated cytoskeletal activation, TCR phosphorylation, and PTEN inactivation, respectively. Cbl-b also directly ubiquitinates SMAD7, promoting TGF-ß signaling and an overall immunosuppressive phenotype.[8] In the event of successful CD28 ligation, however, Cbl-b itself is ubiquitinated and tagged for lysosomal degradation to prevent this anergic response.

The precise mechanism by which CTLA-4 stimulation leads to Cbl-b activation has not yet been elucidated, but several studies have shown a strong correlation between CTLA-4 ligation and both the function and expression of Cbl-b.[51] Furthermore, CTLA-4 signaling leads to increased expression of both Itch and GRAIL, initiating a host of ligase-mediated inhibitory activity.[4] In addition to CD8^{+} T cell effects, CTLA-4 receptor ligation has been

associated with T_{reg} activation—preliminary studies show that GRAIL and associated ligases may be key, downstream mediators of T_{reg} development.[52] Overall, given its critical role in the CD28, CTLA-4, and PD(L)1 pathways, Cbl-b may be tied to both the success of checkpoint blockade along with a possible source of resistance.

The effect of Cbl-b on immune cells
T cells

A number of KO models have been developed to elucidate the effect of Cbl-b signaling in innate and adaptive immune cells. Starting with T cell differentiation, Cbl-b-deficient mice demonstrate increased T_h2, T_h9, and T_h17 cellular differentiation.[53] Phenotypic traits related to this differentiation included a hyperacute immune response secondary to increased JAK/Stat6 signaling in the absence of Cbl-b mediated ubiquitination.[53]

Cbl-b$^{-/-}$ murine models demonstrate a stark response to both infections and malignancy. For example, in the context of lymphocytic choriomeningitis virus (LCMV) infection in Cbl-b KO mice, CD8$^+$ TCR downregulation is limited with increased levels of IFN-γproduction.[54] While Cbl-b inhibition has been shown to be an effective adjunct for inactivated vaccines, complete deficiency has also led to cytokine-storm mediated fatalities in certain murine KO models.[55] Notably, however, Cbl-b knockout models only develop detrimental autoimmune effects on stimulation or after a significant time period, whereas CTLA-4$^{-/-}$ murine models often develop spontaneous, lethal autoimmunity at an early age.[56]

The response of CD8$^+$ T cells in Cbl-b$^{-/-}$ mice exposed to cancer is also enhanced. In a UVB-induced tumor model, over 80% of Cbl-b$^{-/-}$ mice spontaneously rejected tumors as compared with wild-type models.[55] Further murine experiments also showed that adoptively transferred Cbl-b$^{-/-}$ CD8$^+$ T cells could eradicate TC-1 injected tumor cells while promoting ongoing CD8+ T cell proliferation.[55] Similarly, Cbl-b-deficient CAR T cells engineered against carcinoembryonic antigen demonstrated reduced exhaustion markers (PD1$^+$TIM3$^+$) in MC38 syngeneic mice.[57] Additional mechanistic studies have identified IL-9 and Th9 cells as key mediators of antitumor efficacy in Cbl-b$^{-/-}$ models.[58] These results not only reveal the unique role of Cbl-b in defining the cytokine profile and cellular differentiation of the TME, but also support the translational potential of Cbl-b inhibition with cellular therapies.

T_{reg} and myeloid-derived suppressor cells

T_{reg} and myeloid-derived suppressor cells (MDSC) protect against autoimmunity in the setting of chronic antigenic presentation, but in the TME, these cells can lead to anergy, ICI resistance, and increased tumor proliferation. Cbl-b promotes peripheral T_{reg} development via Akt2, converting CD4$^+$CD25$^-$ cells into CD4$^+$CD25$^+$FoxP3$^+$ T cells.[59] In contrast, Cbl-b deficient CD8$^+$ T cells are resistant to T_{reg} or TGF-β-mediated suppression.[59] While the immunosuppressive effects of MDSCs are less well characterized, connections have been identified between the glycolipid, alpha-galactosylceramide (aGalCer), with both Cbl-b activation and MDSC induction.[60] aGalCer has been shown to upregulate Erg2/3 with subsequent induction of PD1 and Cbl-b in NKT cells along with MDSC stimulation.[60]

Natural killer cells

Natural killer (NK) cells directly promote cytotoxicity against stressed and foreign cells in the absence of MHC-II or antibody signaling.[61] Unlike T cells, Cbl-b deficiency does not impact NK differentiation, however Cbl-b$^{-/-}$ mice display markedly enhanced NK proliferation and IFN-γ production.[62] This may be regulated by TAM kinases receptors, ubiquitylation targets of Cbl-b. A small molecule TAM modulator has been shown to significantly reduce metastatic tumor growth in a melanoma murine model, a finding reversed on NK cell depletion.[62] Based on this model, a TAM/Cbl-b inhibitory pathway rendering NK cells dysfunctional has been proposed. Additionally, elucidation of intracellular signaling revealed that NK cell anergy can be induced by Cbl-b mediated ubiquitination of CARMA1.[63] On targeting CARMA1, a component of the CBM complex, Cbl-b ultimately blocks NF-kB transcriptional activity leading to NK cell suppression. Given these data, and the importance of NK-mediated cytotoxicity in the TME, therapeutic Cbl-b inhibition may offer a multicellular approach toward antitumor immunity.

B cells

While humoral-mediated adaptative immunity is not the primary defense against tumor invasion, B cells play a critical role via antigen presentation and cytokine signaling. CD40 is a key costimulatory receptor that leads to isotype switching, germinal cell formation, and NF-kB-mediated proliferation in B cells.[64] Cbl-b induced TRAF signaling inhibits B cell activation in the absence of CD40 activation.[65] Unsurprisingly then, Cbl-b deficiency can significantly enhance CD40-mediated B cell proliferation.[66] Akin to the TCR and CD28 coreceptor on T cells, the simultaneous activation of the BCR and CD40 on B cells leads to a high-affinity, antigen-specific clonal expansion.[6] Cbl-b inhibition lowers the threshold of BCR induced activation regardless of CD40 costimulation.

Dendritic cells

Macrophages and DCs preferentially target and phagocytose microbial antigens or stressed cells via generalized danger sensing receptors (eg, Toll-like receptors, TLR).[67] This rapid but non-specific process recruits additional immune cells and activates the adaptive immune system via antigen presentation using MHC-II receptors.[68] Similar to NK cells, Cbl-b deficiency does not affect DC differentiation. However, Cbl-b$^{-/-}$ mice produced significantly increased levels of TNF-α and IL-6 on LPS-induced TLR4 stimulation as compared with wild-type mice.[8] As opposed to Cbl-b deficient T cells, the enhanced cytokine production of Cbl-b$^{-/-}$ DCs alone does not suffice to prime antigen-specific CD8$^+$ T cell expansion. Additionally,

Cbl-b-deficient mice exposed to LPS, an innate ligand to TLRs, were shown to be highly susceptible to septic shock.[69] Overall, these results clarify the lack of benefit for systemic Cbl-b blockade in the absence of a primary antigenic presentation to adaptive lymphocytes.

Targeting CD28 and Cbl-b in the preclinical setting

The CD28 pathway remains a high priority target for the next generation of IO therapies for several reasons: (1) CD28 ligation is the primary costimulatory signal required for effector T cell response[70]; (2) CD28 agonists have been shown to lower the threshold for T cell stimulation[17]; (3) CD28 receptors are constitutively active in a majority of $CD4^+$ and $CD8^+$ T cells, as opposed to other costimulatory receptors[14]; (4) CTLA-4 inhibitors have been shown to boost CD8+ T cell activity and promote clinical response[71]; and (5) a critical downstream protein, Cbl-b, has been shown to regulate multiple costimulatory and coinhibitory pathways, T cell transcriptional activity, and ultimate immune cell response.[16]

Though certainly promising, numerous challenges have surfaced in the effort to target the CD28 pathway. First, early clinical data demonstrated the risk of highly morbid, autoimmune effects following non-specific agonism of this constitutively active coreceptor.[17] Additionally, extracellular blockade of CTLA-4 alone has had limited success, likely secondary to downstream resistance or bypass mechanisms.[72] Further, T cell infiltration itself is limited by immunosuppressive cytokine (eg, TGF-ß) and cellular (eg, Treg, MDSC) profiles along with poor neoantigenicity.[73] Finally, many downstream molecular mediators (ie, Cbl-b) have been labeled 'undruggable' targets due to unfavorable chemical structures, competitive active sites, and elusive allosteric pockets.[16 55 74]

Because $Cbl-b^{-/-}$ CD8+ T cells have shown resistance to T_{reg} and TGF-β mediated suppression, all while preserving antigenic specificity,[75] multiple groups have pursued novel platforms with the intent of developing clinical therapeutics that inhibit Cbl-b (table 2). Pharmaceutical approaches include DNA encoded libraries and machine learning algorithms, CRISPR genome editing combined with adoptive cell therapy, and siRNA knockdown, among others.

The most advanced Cbl-b targeting program has been developed by Nurix, utilizing a priority DNA encoded library screening platform toward identifying compounds that can link E3 ligases with proteins of interest.[76] This process can target proteins for ligase mediated ubiquitination and ultimately proteasomal destruction. NX-1607 is an oral small molecule that significantly reduces tumor growth in colon and triple negative breast cancer murine models.[77] Cellular and molecular correlates of this inhibition demonstrate enhanced NK and CD8+ T cell activity in the TME; additionally, NX-1607 mediated tumor inhibition was reversed on NK or $CD8^+$ T cell depletion in these models.[77] Given a reduction in TCR diversity and tumor reactive TIL following checkpoint blockade, Nurix has also developed an ex vivo Cbl-b inhibitor (NX-0255) to be used alongside adoptive T cell therapy. This platform, termed drug enhanced tumor infiltrating lymphocyte (DeTIL) therapy, cultures patient-derived tumor fragments with IL-2 and NX-0255 to further stimulate antigen-specific CD8+ T cells. These DeTILs are then reinfused

Table 2 Platforms targeting Cbl-b currently in development or under investigation

Drug/platform	Pharmaceutical	Development and mechanism of action	Investigational stage
Platforms targeting Cbl-b			
NX-1607	Nurix	Computational approach using DEL identified novel, small molecule Cbl-b inhibitor	Phase I NCT05107674
DeTIL-0255		Surgically derived TIL cultured with IL-2 plus Cbl-b inhibitor (NX-0255), then infused as adoptive TIL therapy	Phase I NCT05107739
APN-401	invIOs/Apeiron	CBLB specific siRNA transfected into peripheral leukocytes via electroporation and infused back into patient	Phase I NCT03087591 NCT02166255
bbT 369	2senventy bio	Enhanced cell therapy via CD79a/CD20 CAR-T with CBLB knockout	Phase I NCT05169489
HST-1011	HotSpot	Computational approach identified allosteric regulatory sites on Cbl-b; DEL used to identify specific allosteric inhibitor	Preclinical
NTX-801	Nimbus	Structure-based drug design identified small molecule Cbl-b inhibitor; prevents phosphorylation and activation	Preclinical
Protein Targeting Chimeras (PROTAC), NOS	Progenra	High-throughput screening campaign using a homogenous TR-FRET based Tyro3 substrate ubiquitylation assay to develop hetero-bifunctional small molecules that can bind an E3 ligase±other protein for degradation	Preclinical
STX-500	Synthex	Degrader, NOS	Preclinical
APN-411	invIOs/Apeiron	Small molecule inhibitor, NOS	Preclinical
CD70	Nkarta	CD70 CAR-NK w/ CBLB/CISH CRISPR knockout	Preclinical
NOS	Sanofi	CRISPR gene editing platform in T cells, NOS	Preclinical
NOS	Juno/Celgene/BMS	CRISPR gene editing platform in T cells, NOS	Preclinical

Cbl-b, Casitas B lymphoma-b; DEL, DNA-encoded library; DeTIL, drug enhanced tumor infiltrating lymphocyte; NOS, not otherwise specified.

back into the patient. Both NX-1607 (NCT05107674) and DeTIL-0255 (NCT05107739) have started recruitment efforts for phase I clinical trials in advanced malignancies.

Applying a siRNA platform, Apeiron is combining adoptive T cell therapy with novel Cbl-b inhibition. The APN401 approach uses electroporation to transfect peripheral leukocytes with Cbl-b specific siRNA, and reinfuses these transfected cells back into the patient. While not as tumor specific as DeTIL therapy, APN401 forfeits cellular specificity for the benefits of a completely ambulatory treatment model. Preclinical data assessing siRNA transfected CD8+ T cells alongside a DC-based tumor vaccine showed enhanced intratumoral inflammatory cytokine signaling along with augmented tumor inhibition in a B16 murine model.[78] Preliminary data from a phase I clinical trial, however, showed only marginal clinical improvements—best tumor response entailed four of sixteen patients with stable disease.[79] Regardless, a lack of immune-related adverse events (irAE) has encouraged ongoing translational work with this platform.

Using allosteric chemistry, HotSpot Therapeutics has developed a series of small molecule Cbl-b inhibitors.[80] HotSpot has described a novel regulatory pocket on Cbl-b and developed an inhibitor (HOT-A) that prevents Cbl-b phosphorylation and functional activity.[81] In a series of stepwise experiments, HOT-A was shown to increase IFNγ secretion and CD8+ T cell proliferation using human-derived peripheral blood mononuclear cells. Additionally, K562 tumor cells were killed on coadministration of HOT-A with NK cells. Finally, anti-CD3 treated mice showed enhanced IL-2 secretion and increased differentiation of activated (CD3$^+$/CD69$^+$) T cells on HOT-A delivery.[81] These results, though preliminary, provide a promising foundation for upcoming clinical trials (NCT05662397).

Clinical implications and next steps

The data presented above suggest that Cbl-b, part of the CD28 pathway, is a key mediator of immunosuppression in the TME. Thus, this intracellular ligase may represent a novel and important target for the next phase of IO. To effectively translate the known biology of Cbl-b into clinic, however, focus should be placed on unexplored spaces based on the development of anti-CTLA-4 therapy, identifying biomarkers for optimal patient selection, assessing appropriate combinatorial strategies, and anticipating irAEs.

CD28 and CTLA-4 control Cbl-b expression and activity in a potent but inverse fashion.[51] While CD28 activation inhibits Cbl-b-mediated immunosuppression, CTLA-4 ligation promotes Cbl-b activity, TGF-β signaling, loss of PTEN inactivation, and ultimate T cell anergy (figure 1B).[82] The efficacy of CTLA-4 blockade is dependent on these downstream effects, however, until recently, targeting these intracellular mediators for ICI augmentation has been limited. While anti-CTLA-4 therapy quickly revolutionized the treatment of some advanced cancers after the Food and Drug Administration (FDA) approval of

ipilimumab in 2011, CTLA-4 blockade was soon overshadowed by PD(L)1 inhibitors given significantly improved survival and less toxicity with anti-PD1 versus anti-CTLA-4 monotherapy.[83] Currently, ipilimumab is primarily used in combination with PD1 blockade as induction therapy in select cancers, while anti-PD1 therapy is approved in an ever-growing list of advanced malignancies.[84]

Whereas the differential clinical outcomes between CTLA-4 and PD1 blockade are relatively clear, the cellular and molecular mechanisms explaining this distinction are less well understood. This lack of understanding in pharmacological variation, along with favorable toxicity profile of PD1 monotherapy, may have led to a premature dismissal in targeting the CTLA-4 pathway. Tumors exposed to PD1 antibodies exhibit increased levels of cytolytic transcription factors along with decreased T_{reg} infiltration as compared with CTLA-4 blockade.[85] Additional cellular correlates of therapeutic benefit in patients with melanoma suggest that changes in NK cell subsets may differentiate response to anti-PD1 therapy whereas anti-CLTA-4 response rely primarily on CD4$^+$ and CD8$^+$ memory T-cell subsets.[86] Though further characterization of these independent pathways is still needed, dual checkpoint blockade has been shown to prevent resistance to anti-PD1 therapy caused by compensatory upregulation of secondary inhibitory receptors, a mechanistic explanation behind both augmented clinical benefit as well as increased irAEs.[87] Despite focus on anti-PD1(L)1, several clinical trials have suggested an ongoing role for CTLA-4 inhibitors in multiple cancers, particularly as an immunologic primer alongside PD1 therapy. For example, ipilimumab was shown to improve PFS (4.0 vs 3.1 months, p<0.01) but fell short demonstrating an improvement in OS (11.2 vs 10.0 months, p=0.053) in castration-resistant prostate cancer.[88] In a phase II trial of recurrent ovarian cancer, the addition of ipilimumab to nivolumab resulted in improved and clinically relevant 6 month PFS relative to PD1 monotherapy (25.5% vs 16.3%, p=0.13), though the study was not powered to confirm this difference.[89] Results from CheckMate 227 confirmed OS advantage with ipilimumab plus nivolumab versus chemotherapy as first-line treatment for advanced NSCLC, regardless of PD-L1 expression (median OS 17.1 vs 13.9 mos, HR 0.73, 95% CI 0.64 to 0.84). Notably, the phase III HIMALAYA trial led the FDA to accept tremelimumab under priority review for the treatment of unresectable HCC alongside durvalumab (HR 0.78, p=0.0035, vs sorafenib alone). Finally, ipilimumab has been shown to have some salvage treatment potential in PD1-refractory advanced RCC, providing an immunotherapeutic 'boost' on progression with nivolumab monotherapy.[90] Overall, further investigation and targeting of the CD28, CTLA-4, and Cbl-b pathways may provide a key route toward effective treatment of PD1-resistant tumors.

Biomarkers for Cbl-b therapeutics

Biomarker development remains an area of unmet need in IO and may be of particular relevance surrounding

therapeutic targeting of Cbl-b. PD-L1 expression, tumor mutational burden (TMB), and CD8^{+} T cell infiltration are widely used clinical biomarkers for ICI efficacy, though predictive utility has been inconsistent in many cancers.[91] Gene signatures have been developed to characterize the transcriptional landscape of responding versus non-responding tumors. For example, a T cell-inflamed signature (Tinfl) containing IFN-γ responsive genes has shown predictive value in multiple cancers treated with anti-PD1 therapy.[92] Additionally, an adenosine signature (AdenoSig) containing myeloid and inflammatory related genes was found to predict tumor regression

on anti-adenosine therapy.[93] Thus, pretreatment gene expression profiles might be useful in identifying patients who could benefit from anti-Cbl-b therapy. As a preliminary assessment, we postulate that patient tumors with low/moderate Tinfl expression, low/moderate TMB, and high CBLB expression might be a high unmet need population who could benefit from Cbl-b inhibition. Cancers historically unresponsive to ICI, for example, CRC, ovarian, and prostate, can be classified into Tinfl[lo]/CBLB[hi] tumors based on expression data (figure 2A). Notably, the pharmacokinetic penetration of small molecule Cbl-b inhibitors into the TME may increase their
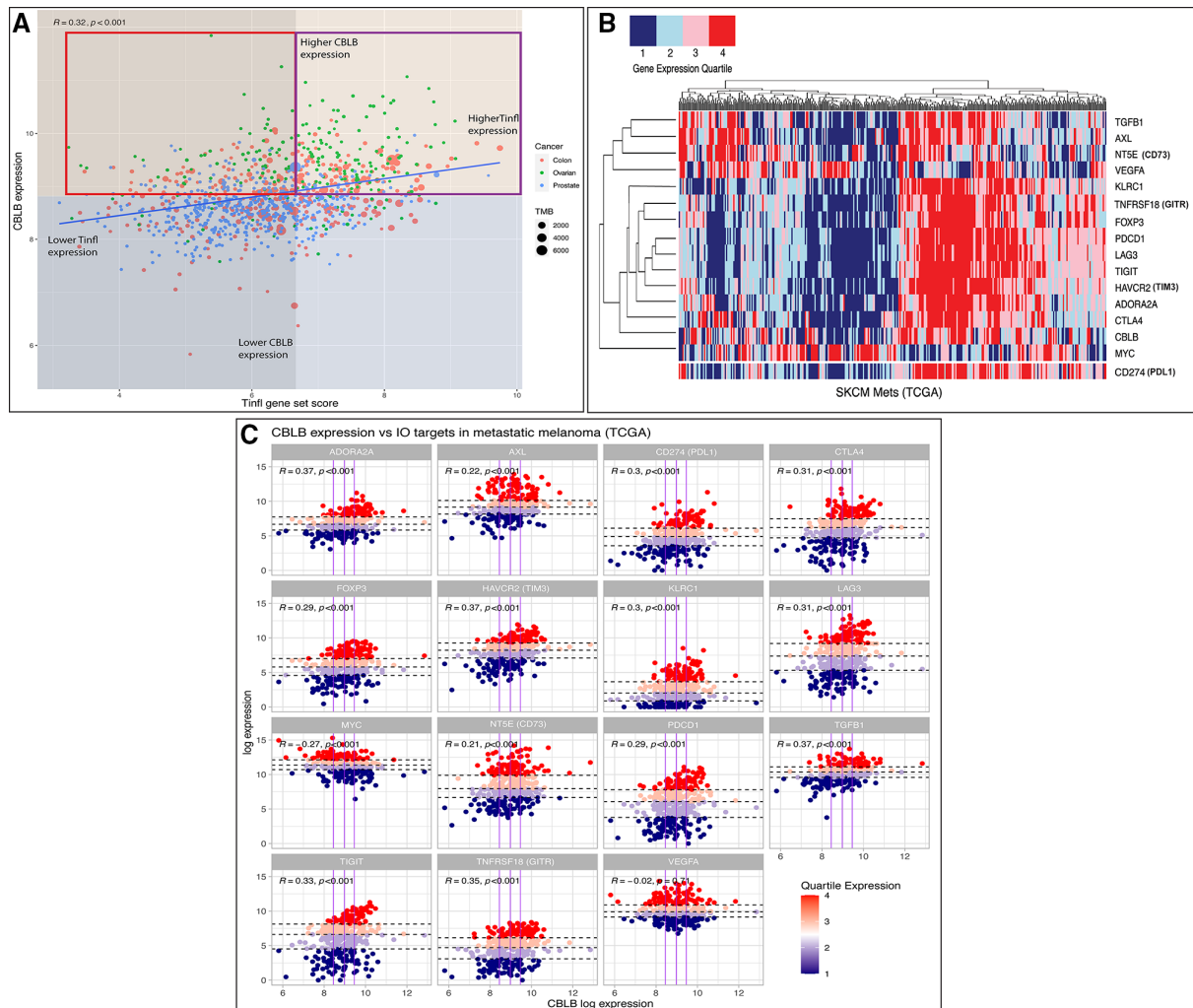
Figure 2 (A) Comparison of T cell-inflamed gene set enrichment score (Tinfl)[100] versus *CBLB* gene expression across select TCGA tumor samples. Red box highlights tumor samples postulated to receive greatest benefit from Cbl-b inhibition versus ICI therapy±Cbl-b inhibition (purple box). Median expression values denoted by orange (higher *CBLB*), blue (lower *CBLB*), gray (lower Tinfl), and transparent (higher Tinfl). TMB, tumor mutational burden. Spearman's correlation coefficient and p value are shown at the left corner of the figure. Methods: Gene set enrichment analysis (GSEA) was performed using normalized and log2-transformed RNAseq gene expression data from the TCGA. All analyses were performed using Bioconductor packages in R (V.4.0.3).[100] (B) Heatmap of various IO targets (right) based on quartile expression of TCGA metastatic melanoma samples. CD274 (PDL1) data were added to bottom of heatmap for visual reference. Methods: Unsupervised hierarchical clustering performed across tumor samples; quartile expression calculated across all samples per gene. All analyses were performed using R (V.4.0.3).[100] (C) Expression of fifteen IO targets versus *CBLB* expression in TCGA metastatic melanoma samples. Vertical (purple) lines denote *CBLB* quartiles; horizontal (dashed) lines denote IO target quartiles per facet. Methods: Faceted scatterplot with colors denoting quartile expression of each gene in R (V.4.0.3). Cbl-b, Casitas B lymphoma-b; ICI, immune checkpoint inhibitor; IO, immuno-oncology.

effectiveness in TMB^{lo}/TIL^{lo} tumors as compared with traditional checkpoint antibodies. Overall, the immunostimulatory properties of this therapy, IO non-responsive nature of these tumors, and lack of effective options for this cohort could provide useful guidance for clinical application. Integrating additional biomarkers, including microsatellite instability, mismatch repair deficiency, and TCR diversity, may offer further predictive power in the future.

Further analyzing the expression data of melanoma samples reveals that CBLB clusters with other IO targets and associates with PDL1 expression, indicating a unified phenotype among T cell-inflamed tumors (figure 2B).[94] As shown in figure 2C, this pattern offers potential strategies for combinatorial therapies. For example, TGFβ1 expression is correlated with both CBLB and PDL1, denoting a similar profile to the aggressive CRC mesenchymal subtype (CMS4).[95] This molecular subtype is characterized by increased stromal invasion, moderate immune infiltration scores, and upregulation of TGF-β, prompting novel drug development and ongoing clinical trials targeting the TGF-β pathway.[96] The exceptions to this pattern, however, suggest other potential strategies for treating non-T cell-inflamed tumors. Figure 2B,C shows that VEGFA and MYC do not correlate with either PD-L1 or CBLB. Robust preclinical work has led to numerous clinical trials aiming to 'normalize' the chaotic vasculature of the TME and promote immune cell infiltration using anti-VEGF priming plus checkpoint blockade.[97] Similarly, the MYC family of transcription factors has long been implicated in decreasing the immunogenicity of the TME, yet targeting this master regulator and its downstream processes has remained elusive due to its pharmacologic challenges, not unlike Cbl-b.[98] Overall, further *in silico* studies of gene signatures will continue to offer strategies to augment existing ICI, reverse the immunosuppressive TME, and expose novel IO targets.

While Cbl-b has been most frequently studied in the context of CD28 and CTLA-4, other relevant associations have been identified with pathways such as PD1/PD-L1 signaling.[99] A B16 melanoma model resistant to PD-L1 mediated suppression showed significant tumor regression with Cbl-b$^{-/-}$ T cells.[99] These results highlight Cbl-b as a key downstream mediator of PD1 signaling that could also serve as a potential source of anti-PD1 resistance and emphasize the potential for an augmented benefit of combinatorial anti-PD1 plus Cbl-b blockade. Future studies comparing the mutations, expression, and functional levels of Cbl-b in anti-PD1 responsive versus nonresponsive tumors would provide further clarification. Additional elements leading to immunosuppression in the TME include certain metabolites, vascular components, and other T cell coreceptors. Ongoing trials targeting these immunological mediators alongside ICI may help identify unmet needs in non-responding cohorts, predict the utility of combinatorial strategies with Cbl-b blockade, and highlight potential patient populations most likely to benefit.

As described with the novel treatment platforms, combining Cbl-b inhibition with tumor specific T cells will be a key element in the effort to maximize site specific immune stimulation and limit irAEs. Adoptive T cell transfer and antibody-drug conjugates provide routes for tumor specific drug delivery. While the former specifically addresses T cell infiltration, the latter may provide a more feasible and less intensive treatment algorithm for delivering Cbl-b inhibitors to the TME. Regardless, the dichotomy between tumor-specific targets and systemic immune activation is not a new IO-related principle, but one that remains relevant for achieving clinical benefit while minimizing dose-related toxicities.

Cbl-b is a downstream, master regulator of both costimulatory and coinhibitory pathways of T cell activation—thus representing a prime target to reverse immune suppression in the TME. With the aid of novel biopharmaceutical platforms, Cbl-b inhibitors can now target this once 'undruggable' downstream mediator of CD28/CTLA-4 signaling, provide immune stimulation in the context of antigen specificity, offer antitumor activity in cancers unresponsive to established checkpoint therapy, and could soon be added to the armamentarium of IO.

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REFERENCES

1 Li X, Shao C, Shi Y, *et al*. Lessons learned from the blockade of immune checkpoints in cancer immunotherapy. *J Hematol Oncol* 2018;11:31.
2 Pitt JM, Marabelle A, Eggermont A, *et al*. Targeting the tumor microenvironment: removing obstruction to anticancer immune responses and immunotherapy. *Ann Oncol* 2016;27:1482–92.
3 Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol* 2013;13:227–42.
4 Gavali S, Liu J, Li X, *et al*. Ubiquitination in T-cell activation and checkpoint inhibition: new avenues for targeted cancer immunotherapy. *Int J Mol Sci* 2021;22:10800:19.:.
5 O'Neill RE, Cao X. Co-stimulatory and co-inhibitory pathways in cancer immunotherapy. *Adv Cancer Res* 2019;143:145–94.
6 Liu Q, Zhou H, Langdon WY, *et al*. E3 ubiquitin ligase cbl-b in innate and adaptive immunity. *Cell Cycle* 2014;13:1875–84.
7 Emens LA, Butterfield LH, Hodi FS, *et al*. Cancer immunotherapy trials: leading a paradigm shift in drug development. *J Immunother Cancer* 2016;4:42.
8 Lutz-Nicoladoni C, Wolf D, Sopper S. Modulation of immune cell functions by the E3 ligase cbl-b. *Front Oncol* 2015;5:58.
9 Chiang YJ, Kole HK, Brown K, *et al*. Cbl-b regulates the CD28 dependence of T-cell activation. *Nature* 2000;403:216–20.
10 Spranger S. Mechanisms of tumor escape in the context of the T-cell-inflamed and the non-T-cell-inflamed tumor microenvironment. *Int Immunol* 2016;28:383–91.
11 Schnell A, Bod L, Madi A, *et al*. The yin and yang of co-inhibitory receptors: toward anti-tumor immunity without autoimmunity. *Cell Res* 2020;30:285–99.
12 Bour-Jordan H, Esensten JH, Martinez-Llordella M, *et al*. Intrinsic and extrinsic control of peripheral T-cell tolerance by costimulatory molecules of the CD28/ B7 family. *Immunol Rev* 2011;241:180–205.
13 Linsley PS, Greene JL, Brady W, *et al*. Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. *Immunity* 1994;1:793–801.
14 Rudd CE, Taylor A, Schneider H. Cd28 and CTLA-4 coreceptor expression and signal transduction. *Immunol Rev* 2009;229:12–26.
15 Yang B, Gay DL, MacLeod MKL, *et al*. Nedd4 augments the adaptive immune response by promoting ubiquitin-mediated degradation of Cbl-b in activated T cells. *Nat Immunol* 2008;9:1356–63.
16 Tang R, Langdon WY, Zhang J. Regulation of immune responses by E3 ubiquitin ligase cbl-b. *Cell Immunol* 2019;340:103878.
17 Hünig T. The storm has cleared: lessons from the CD28 superagonist TGN1412 trial. *Nat Rev Immunol* 2012;12:317–8.
18 Vinay DS, Kwon BS. Immunotherapy of cancer with 4-1BB. *Mol Cancer Ther* 2012;11:1062–70.
19 Sznol M, Hodi FS, Margolin K, *et al*. Phase I study of BMS-663513, a fully human anti-CD137 agonist monoclonal antibody, in patients (PTS) with advanced cancer (ca). *JCO* 2008;26(15_suppl):3007.
20 Li S-Y, Liu Y. Immunotherapy of melanoma with the immune costimulatory monoclonal antibodies targeting CD137. *Clin Pharmacol* 2013;5(Suppl 1):47–53.
21 Garralda E, Geva R, Ben-Ami E, *et al*. 412 First-in-human phase I/iia trial to evaluate the safety and initial clinical activity of duobody®-PD-L1×4–1BB (GEN1046) in patients with advanced solid tumors. 35th Anniversary Annual Meeting (SITC 2020); November 2020
22 Luke JJ, Saeed A, Bashir B, *et al*. Phase 1 dose escalation study of DSP107, a first-in-class CD47 and 4-1BB targeting multifunctional immune-recruitment protein, in patients with advanced solid tumors. *JCO* 2022;40(16_suppl):2647.
23 Glisson BS, Leidner RS, Ferris RL, *et al*. Safety and clinical activity of MEDI0562, a humanized OX40 agonist monoclonal antibody, in adult patients with advanced solid tumors. *Clin Cancer Res* 2020;26:5358–67.
24 Gutierrez M, Moreno V, Heinhuis KM, *et al*. OX40 agonist BMS-986178 alone or in combination with nivolumab and/or ipilimumab in patients with advanced solid tumors. *Clin Cancer Res* 2021;27:460–72.
25 Wang R, Gao C, Raymond M, *et al*. An integrative approach to inform optimal administration of OX40 agonist antibodies in patients with advanced solid tumors. *Clin Cancer Res* 2019;25:6709–20.
26 Buzzatti G, Dellepiane C, Del Mastro L. New emerging targets in cancer immunotherapy: the role of GITR. *ESMO Open* 2020;4(Suppl 3):e000738.
27 ClinicalTrials.gov. Phase 1/2 study exploring the safety, tolerability, and efficacy of INCAGN01876 combined with immune therapies in advanced or metastatic malignancies. Available: https://clinicaltrials.gov/ct2/show/NCT03126110 [Accessed 22 Nov 2021].
28 ClinicalTrials.gov. Biomarker-driven therapy using immune activators with nivolumab in patients with first recurrence of glioblastoma. Available: https://clinicaltrials.gov/ct2/show/NCT03707457 [Accessed 22 Nov 2021].
29 Davar D, Zappasodi R, Wang H, *et al*. Phase Ib study of GITR agonist antibody TRX518 singly and in combination with gemcitabine, pembrolizumab, or nivolumab in patients with advanced solid tumors. *Clin Cancer Res* 2022;28:3990–4002.
30 Heinhuis KM, Carlino M, Joerger M, *et al*. Safety, tolerability, and potential clinical activity of a glucocorticoid-induced TNF receptor-related protein agonist alone or in combination with nivolumab for patients with advanced solid tumors: a phase 1/2A dose-escalation and cohort-expansion clinical trial. *JAMA Oncol* 2020;6:100–7.
31 Paulos CM, Carpenito C, Plesa G, *et al*. The inducible costimulator (ICOS) is critical for the development of human T (H) 17 cells. *Sci Transl Med* 2010;2:55.
32 Burris HA, Callahan MK, Tolcher AW, *et al*. Phase 1 safety of ICOS agonist antibody JTX-2011 alone and with nivolumab (nivo) in advanced solid tumors; predicted vs observed pharmacokinetics (pk) in iconic. *JCO* 2017;35(15_suppl):3033.
33 GSK. GSK provides update on feladilimab, an investigational inducible T cell co-stimulatory (ICOS) agonist. Available: https://www.gsk.com/en-gb/media/press-releases/gsk-provides-update-on-feladilimab-an-investigational-inducible-t-cell-co-stimulatory-icos-agonist [Accessed 4 Jan 2022].
34 Starzer AM, Berghoff AS. New emerging targets in cancer immunotherapy: CD27 (TNFRSF7). *ESMO Open* 2020;4(Suppl 3):e000629.
35 Sanborn RE, Pishvaian MJ, Callahan MK, *et al*. Anti-cd27 agonist antibody varlilumab (varli) with nivolumab (nivo) for colorectal (CRC) and ovarian (ova) cancer: phase (ph) 1/2 clinical trial results. *JCO* 2018;36(15_suppl):3001.
36 Kamphorst AO, Wieland A, Nasti T, *et al*. Rescue of exhausted CD8 T cells by PD-1-targeted therapies is CD28-dependent. *Science* 2017;355:1423–7.
37 Waite JC, Wang B, Haber L, *et al*. Tumor-Targeted CD28 bispecific antibodies enhance the antitumor efficacy of PD-1 immunotherapy. *Sci Transl Med* 2020;12:eaba2325:549.:.
38 Zhang J, Stein MN, Kelly WK, *et al*. A phase I/II study of REGN5678 (anti-psmaxcd28, A costimulatory bispecific antibody) with cemiplimab (anti–PD-1) in patients with metastatic castration-resistant prostate cancer. *JCO* 2021;39(6_suppl):TPS174.
39 Haber L, Olson K, Kelly MP, *et al*. Generation of T-cell-redirecting bispecific antibodies with differentiated profiles of cytokine release and biodistribution by CD3 affinity tuning. *Sci Rep* 2021;11:14397.
40 ClinicalTrials.gov. Dose escalation study of TAB08 in patients with advanced solid neoplasms. Available: https://clinicaltrials.gov/ct2/show/NCT03006029 [Accessed 23 Nov 2021].
41 Haile ST, Dalal SP, Clements V, *et al*. Soluble CD80 restores T cell activation and overcomes tumor cell programmed death ligand 1-mediated immune suppression. *J Immunol* 2013;191:2829–36.
42 Barbee SD, Sennino B, De La Torre J, *et al*. Abstract B005: FPT155, a novel therapeutic CD80-fc fusion protein with potent antitumor activity in preclinical models. *Molecular Cancer Therapeutics* 2018;17(1_Supplement):B005.
43 Davar D, Moser JC, Millward M, *et al*. Dose escalation of davoceticept, a conditional CD28 costimulator and dual checkpoint inhibitor, in advanced malignancies (neon-1). *JCO* 2022;40(16_suppl):2560.
44 Patnaik A, Lakhani NJ, McKean M, *et al*. Davoceticept (ALPN-202), a PD-L1-dependent CD28 costimulator and dual checkpoint inhibitor, in combination with pembrolizumab in patients with advanced malignancies (NEON-2). *JCO* 2022;40(16_suppl):TPS2683.
45 Sun J-Y, Zhang D, Wu S, *et al*. Resistance to PD-1/PD-L1 blockade cancer immunotherapy: mechanisms, predictive factors, and future perspectives. *Biomark Res* 2020;8:35.
46 Brunner-Weinzierl MC, Rudd CE. Ctla-4 and PD-1 control of T-cell motility and migration: implications for tumor immunotherapy. *Front Immunol* 2018;9:2737.
47 Arasanz H, Gato-Cañas M, Zuazo M, *et al*. Pd1 signal transduction pathways in T cells. *Oncotarget* 2017;8:51936–45.
48 Shin DS, Zaretsky JM, Escuin-Ordinas H, *et al*. Primary resistance to PD-1 blockade mediated by JAK1/2 mutations. *Cancer Discov* 2017;7:188–201.
49 Clark RB. Cbl-b deficiency renders T cells resistant to PD-L1. *The Journal of Immunology* 2016;196:1.

50 Karwacz K, Bricogne C, MacDonald D, *et al*. Pd-L1 co-stimulation contributes to ligand-induced T cell receptor down-modulation on CD8+ T cells. *EMBO Mol Med* 2011;3:581–92.

51 Li D, Gál I, Vermes C, *et al*. Cutting edge: cbl-b: one of the key molecules tuning CD28- and CTLA-4-mediated T cell costimulation. *J Immunol* 2004;173:7135–9.

52 MacKenzie DA, Schartner J, Lin J, *et al*. GRAIL is up-regulated in CD4+ CD25+ T regulatory cells and is sufficient for conversion of T cells to a regulatory phenotype. *J Biol Chem* 2007;282:S0021-9258(19)33624-5:9696–702.:.

53 Qiao G, Ying H, Zhao Y, *et al*. E3 ubiquitin ligase cbl-b suppresses proallergic T cell development and allergic airway inflammation. *Cell Rep* 2014;6:S2211-1247(14)00029-1:709–23.:.

54 Shamim M, Nanjappa SG, Singh A, *et al*. Cbl-b regulates antigen-induced TCR down-regulation and IFN-gamma production by effector CD8 T cells without affecting functional avidity. *J Immunol* 2007;179:7233–43.

55 Loeser S, Loser K, Bijker MS, *et al*. Spontaneous tumor rejection by cbl-b-deficient CD8+ T cells. *J Exp Med* 2007;204:879–91.

56 Tivol EA, Borriello F, Schweitzer AN, *et al*. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 1995;3:541–7.

57 Kumar J, Kumar R, Kumar Singh A, *et al*. Deletion of Cbl-b inhibits CD8+ T-cell exhaustion and promotes CAR T-cell function. *J Immunother Cancer* 2021;9:e001688.

58 Schanz O, Cornez I, Yajnanarayana SP, *et al*. Tumor rejection in cblb-/- mice ᵈᵉpends on IL-9 and th9 cells. *J Immunother Cancer* 2021:e002889.

59 Qiao G, Zhao Y, Li Z, *et al*. T cell activation threshold regulated by E3 ubiquitin ligase Cbl-b determines fate of inducible regulatory T cells. *J Immunol* 2013;191:632–9.

60 Huang J-R, Tsai Y-C, Chang Y-J, *et al*. Α-Galactosylceramide but not phenyl-glycolipids induced NKT cell anergy and IL-33-mediated myeloid-derived suppressor cell accumulation via upregulation of egr2/3. *J Immunol* 2014;192:1972–81.

61 Vivier E, Tomasello E, Baratin M, *et al*. Functions of natural killer cells. *Nat Immunol* 2008;9:503–10.

62 Paolino M, Choidas A, Wallner S, *et al*. The E3 ligase Cbl-b and TAM receptors regulate cancer metastasis via natural killer cells. *Nature* 2014;507:508–12.

63 Kojo S, Elly C, Harada Y, *et al*. Mechanisms of NKT cell anergy induction involve cbl-b-promoted monoubiquitination of CARMA1. *Proc Natl Acad Sci U S A* 2009;106:17847–51.

64 van Kooten C, Banchereau J. Functions of CD40 on B cells, dendritic cells and other cells. *Curr Opin Immunol* 1997;9:330–7.

65 Krawczyk CM, Jones RG, Atfield A, *et al*. Differential control of CD28-regulated in vivo immunity by the E3 ligase Cbl-b. *J Immunol* 2005;174:1472–8.

66 Qiao G, Lei M, Li Z, *et al*. Negative regulation of CD40-mediated B cell responses by E3 ubiquitin ligase casitas-B-lineage lymphoma protein-B. *J Immunol* 2007;179:4473–9.

67 Liu YJ. Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. *Cell* 2001;106:259–62.

68 Wylie B, Macri C, Mintern JD, *et al*. Dendritic cells and cancer: from biology to therapeutic intervention. *Cancers (Basel)* 2019;11:521.

69 Bachmaier K, Toya S, Gao X, *et al*. E3 ubiquitin ligase cblb regulates the acute inflammatory response underlying lung injury. *Nat Med* 2007;13:920–6.

70 Riha P, Rudd CE. CD28 co-signaling in the adaptive immune response. *Self Nonself* 2010;1:231–40.

71 Mocellin S, Nitti D. CTLA-4 blockade and the renaissance of cancer immunotherapy. *Biochim Biophys Acta* 2013;1836:187–96.

72 Liu D, Jenkins RW, Sullivan RJ. Mechanisms of resistance to immune checkpoint blockade. *Am J Clin Dermatol* 2019;20:41–54.

73 Ziai J, Gilbert HN, Foreman O, *et al*. CD8+ T cell infiltration in breast and colon cancer: A histologic and statistical analysis. *PLoS One* 2018;13:e0190158.

74 Dang CV, Reddy EP, Shokat KM, *et al*. Drugging the "undruggable" cancer targets. *Nat Rev Cancer* 2017;17:502–8.

75 Peer S, Baier G, Gruber T. Cblb-deficient T cells are less susceptible to PD-L1-mediated inhibition. *Oncotarget* 2017;8:41841–53.

76 Clark MA, Acharya RA, Arico-Muendel CC, *et al*. Design, synthesis and selection of DNA-encoded small-molecule libraries. *Nat Chem Biol* 2009;5:647–54.

77 Rountree R, Cohen F, Tenn-McClellan A, *et al*. Abstract 1595: small molecule inhibition of the ubiquitin ligase cbl-b results in potent T and NK cell mediated anti-tumor response. *Cancer Research* 2021;81(13_Supplement):1595.

78 Hinterleitner R, Gruber T, Pfeifhofer-Obermair C, *et al*. Adoptive transfer of siRNA cblb-silenced CD8+ T lymphocytes augments tumor vaccine efficacy in a B16 melanoma model. *PLoS One* 2012;7:e44295.

79 Loibner H, Lametschwandtner G, Westritschnig K, *et al*. Adoptive cellular immunotherapy with APN401, autologous cbl-b silenced peripheral blood mononuclear cells: data from a phase I study in patients with solid tumors. *JCO* 2018;36(15_suppl):3055.

80 Sheik Amamuddy O, Veldman W, Manyumwa C, *et al*. Integrated computational approaches and tools forallosteric drug discovery. *Int J Mol Sci* 2020;21:847.

81 Kuai J, Bi Y, Qi Y, *et al*. 864 identification of a novel allosteric oral cbl-b inhibitor that augmented T cell response and enhanced NK cell killing in vitro and in vivo. *J Immunother Cancer* 2021;9(Suppl 2):A905.

82 Chen W, Jin W, Wahl SM. Engagement of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) induces transforming growth factor beta (TGF-beta) production by murine CD4(+) T cells. *J Exp Med* 1998;188:1849–57.

83 Robert C, Schachter J, Long GV, *et al*. Pembrolizumab versus ipilimumab in advanced melanoma. *N Engl J Med* 2015;372:2521–32.

84 Vaddepally RK, Kharel P, Pandey R, *et al*. Review of indications of FDA-approved immune checkpoint inhibitors per NCCN guidelines with the level of evidence. *Cancers (Basel)* 2020;12:738.

85 Duraiswamy J, Kaluza KM, Freeman GJ, *et al*. Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection function in tumors. *Cancer Res* 2013;73:3591–603.

86 Subrahmanyam PB, Dong Z, Gusenleitner D, *et al*. Distinct predictive biomarker candidates for response to anti-CTLA-4 and anti-PD-1 immunotherapy in melanoma patients. *J Immunother Cancer* 2018;6:18.

87 Wu K, Yi M, Qin S, *et al*. The efficacy and safety of combination of PD-1 and CTLA-4 inhibitors: a meta-analysis. *Exp Hematol Oncol* 2019;8:26.

88 Kwon ED, Drake CG, Scher HI, *et al*. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. *Lancet Oncol* 2014;15:700–12.

89 Zamarin D, Burger RA, Sill MW, *et al*. Randomized phase II trial of nivolumab versus nivolumab and ipilimumab for recurrent or persistent ovarian cancer: an NRG oncology study. *J Clin Oncol* 2020;38:1814–23.

90 Grimm M-O, Schmidinger M, Duran Martinez I, *et al*. Tailored immunotherapy approach with nivolumab in advanced renal cell carcinoma (TITAN-RCC). *Annals of Oncology* 2019;30:v892.

91 Yi M, Jiao D, Xu H, *et al*. Biomarkers for predicting efficacy of PD-1/PD-L1 inhibitors. *Mol Cancer* 2018;17:129.

92 Ayers M, Lunceford J, Nebozhyn M, *et al*. IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest* 2017;127:91190:2930–40.:.

93 Fong L, Hotson A, Powderly JD, *et al*. Adenosine 2A receptor blockade as an immunotherapy for treatment-refractory renal cell cancer. *Cancer Discov* 2020;10:40–53.

94 Ott PA, Bang Y-J, Piha-Paul SA, *et al*. T-cell-inflamed gene-expression profile, programmed death ligand 1 expression, and tumor mutational burden predict efficacy in patients treated with pembrolizumab across 20 cancers: KEYNOTE-028. *J Clin Oncol* 2019;37:318–27.

95 Guinney J, Dienstmann R, Wang X, *et al*. The consensus molecular subtypes of colorectal cancer. *Nat Med* 2015;21:1350–6.

96 Kim B-G, Malek E, Choi SH, *et al*. Novel therapies emerging in oncology to target the TGF-β pathway. *J Hematol Oncol* 2021;14:55.

97 Ramjiawan RR, Griffioen AW, Duda DG. Anti-Angiogenesis for cancer revisited: is there a role for combinations with immunotherapy? *Angiogenesis* 2017;20:185–204.

98 Wang C, Zhang J, Yin J, *et al*. Alternative approaches to target Myc for cancer treatment. *Signal Transduct Target Ther* 2021;6:117.

99 Fujiwara M, Anstadt EJ, Clark RB. Cbl-B deficiency mediates resistance to programmed death-ligand 1/programmed death-1 regulation. *Front Immunol* 2017;8:42.

100 Spranger S, Luke JJ, Bao R, *et al*. Density of immunogenic antigens does not explain the presence or absence of the T-cell-inflamed tumor microenvironment in melanoma. *Proc Natl Acad Sci U S A* 2016;113:E7759–68.