Table S1: Combination Immunotherapy: Small Molecules that Boost Immune Response in Combination with Other Agents

Target	Compound (MOA)	Combination	Model	Observation	Reference
IDO1	NLG919 and indoximod (inhibitor)	Anti-PD-1/PD-L1/PD-L2 mAbs	Murine B16 F10 melanoma	Synergistic compared with single checkpoint inhibition	Mautino, 2014[1]
IDO1	1-MT (inhibitor)	Anti-CTLA4, PD-L1	Murine gloma	Combination blockade targets Tregs and enhances survival from established glioma	Wainwright, 2014[2]
IDO1	1-MT, IDO1 (knockdown)	Anti-CTLA4, PD-L1	Syngeneic murine B16 F10 melanoma	Synergistic efficacy, delayed tumor growth and prolonged survival	Holmgaard, 2014[3]
IDO1	Imatinib (inhibitor)	Anti-CTLA4 mAb	Transgenic GIST murine (KitV558+)	Reduced tumor volume relative to imatinib single agent, not curative	Balachandran, 2011[4]
$A_{2A}R$	SCH58261 (antagonist)	Anti-PD-1 mAb	B16-F10 melanoma and 4T1.2 mammary carcinoma	Combination significantly reduces metastatic burden and prolongs the life of mice compared with monotherapy	Mittal, 2014[5]
$A_{2A}R$	ZM241365 (antagonist)	Anti-CTLA4	Syngeneic murine B16 F10 melanoma	Combination inhibited tumor growth and enhanced antitumor immune responses	Iannone, 2014[6]
CD73	AMPCP (inhibitor)	Anti-CTLA4 mAb	Syngeneic murine B16 F10 melanoma	Combination significantly reduces metastatic burden and delays tumor growth	Iannone, 2014[6]
CD73	AMPCP (inhibitor)	Adpotive T-cell therapy	Murine B16-SIY melanoma	AMPCP but not adoptive therapy slowed tumor growth; combination resulted in tumor regression	Wang, 2011[7]

CD73	CD73 mAb (inhibitor)	Anti-PD-1 and anti-CTLA4 mAbs	MC38-OVA (colon) RM-1 (prostate) and 4T1.2 (breast)	Combination of CD73 mAb significantly enhanced the activity of both anti-CTLA4 and anti-PD1	Allard, 2013[8]
TLR3	Poly-IC (agonist)	Anti-PD-L1 mAb	Murine B16F10 melanoma, lung (LLC)-A9F1 and MC38 colorectal adenocarcinoma	Combination resulted insignificant reduction or complete eradication of tumor; mice resistant to rechallenge	Nagato, 2014[9]
TLR7	R848 (agonist)	Radiation	Murine syngeneic T and B cell lymphoma	Longstanding clearance of tumor; cured mice resistant to rechallenge	Dovedi, 2013[10]
TLR7	Imiquimod (agonist)	IDO1 inhibitor (1-MT)	Murine colon carcinoma CT26	Combination therapy with imiquimod and 1-MT significantly inhibited tumor growth	Ito, 2014[11]
TLR9	CpG 1826 (agonist)	Anti-CTLA4 and anti-OX40	Murine A20 lymphoma	Each antibody alone enhanced efficacy of CpG1826; combination with both mAbs resulted in tumor clearance; mice resistant to tumor upon rechallenge	Houot, 2009[12]
TLR9	CpG (agonist)	Anti-PD-1 and anti-CTLA4 mAbs	Murine experimental bladder cancer	Combination of CpG with CTLA-4 or PD-1 blockade improved long-term survival and led to increased Teffs and decreased Tregs at the tumor site	Mangsbo [13], 2010
TRL9	CpG ODN (agonist)	TLR7/8 agonist (3M-052)	CT26 colon carcinoma, syngeneic murine tumor	Increased activity of immune infiltrate; combinatorial efficacy eradicated primary tumors and established protective immunity	Zhao, 2014[14]
TLR9	C792 (agonist)	Bortezomib	Murine xenograft model of human MM and *ex vivo*	Improved immune function and overcome drug resistance	Ray, 2014[15]

TLR 3/9	Poly(I:C)/CpG (agonist)	Adoptive T cell therapy	Syngeneic murine B16 F10 melanoma	TLR agonist enhanced IFN-g production by adoptively transferred T cells resulting in enhanced tumor immunogenicity and increased tumor killing	Amos, 2011[16]
CXCR2	Anti-CXCR2 mAb	Anti-PD-1 mAb	Rhabdomyosarcoma tumor	Reduced MDSC infiltration	Highfill, 2014[17]
CXCR4	Plerixafor (AMD3100)	Anti-PD-1 and anti-CTLA4 mAbs	KPC model of pancreatic ductal carcinoma	Checkpoint blockers had no effect as single agents, but in combination with plerixafor anti-PD-1 (but not anti-CTLA4) decreased tumor volume	Feig, 2013[18]
BRAF	Vemurafenib (inhibitor)	Anti-PD-1 and anti-PDL1 mAb	Murine syngeneic BRAF(V600E)/Pten-/- tumor	Combination significantly prolonging survival and slowed tumor growth; increased number and activity of TILs	Cooper, 2014[19]
CSF-1	PLX3397 (inhibitor)	Anti-PD-1 and anti-CTLA4 mAbs	Murine pancreatic ductal adenocarcinoma (PDAC)	Checkpoint blockers had no effect as single agents, but in combination with CSF-1R blocker elicited tumor regressions	Zhu, 2014[20]

Footnotes: Abbreviations

1-MT, 1-methyltryptophan; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand (also known as B7 homolog 1 [B7-H1]), AMPCP, adenosine 5'-(α,β-methylene)diphosphate

Legand

Many pre-clinical studies have demonstrated significant, even synergistic, value in combining immune-stimulating SMDs with other therapies. IDO-1 inhibitors have been studied in combination with surrogate T cell checkpoint modulating mAbs to CTLA-4, PD-1, and GITR. In each case, both efficacy and related PD aspects of improved immune activity/function were observed in these studies across a variety of murine models.[1-4] The combination of CD73 inhibitors or receptor A_{2A} pathway antagonists (SMDs or surrogate mAbs) with either PD-1 or CTLA-4 inhibitory surrogate mAbs consistently results in improved efficacy.[5-8-] TLR agonists have been studied extensively pre-clinically and clinically, albeit usually in the context of a vaccine approach with an adjuvant function. In light of the compensatory mechanisms that are engaged to regulate the immune response to TLR agonist, interest has now shifted to combinations of TLR with other agents that will counteract these immuno-

suppressive mechanisms. Several recent reports in murine syngeneic tumor models highlight additional value of TLR agonist in direct combination with other immune-modulating agents including anti PD1L-1 and CTLA4 mAbs and agonist OX40 mAbs[9, 12, 13] or in concert with adoptive T-cell therapy.[16] In these models the combination therapies often result in total clearance of tumors and the "cured" animals are resistant to re-introduction of the tumor. Other reported studies with TLR agonists include combinations with other TLR agonists, IDO-1 inhibitors or radiation and report not only improved (sometimes curative) efficacy in murine models but also demonstrate significant improvements in immune tumor infiltration and activation of T cell function.[10, 11, 14] Emerging evidence also suggests that combining TLR agonists with SoC such as bortezomib for multiple myeloma can result in improved immune function and even overcome drug resistance during treatment.[15] Chemokine antagonists also provide improved efficacy when used with anti-PD-1 mAbs, presumably by blocking access of MDSCs (CXCR2) or by enhancing access of T effector cells (CXCR4) to the TME.[17, 18] Following the recent success of experimental medicines targeting PD-1 & PDL1, pre-clinical[19, 20] and clinical combination studies (Table S2) are underway with signal transduction inhibitors with the expectation of additional immune-activation via their tumor cell killing activity. In summary the combined evidence across both syngeneic and tumor xenograft models provides compelling confidence to enable rational clinical trial design for experimental medicine combination treatments.

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