MAP4K1 inhibition enhances immune cell activation and anti-tumor immunity in preclinical tumor models

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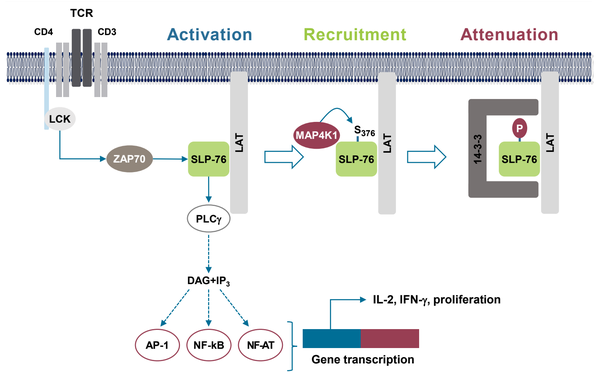
Background

- Hematopoietic progenitor kinase 1 (HPK1, MAP4K1) is a serine/threonine (SER/THR) kinase that has been demonstrated to have suppressive effects across a range of immune cells, including T cells and dendritic cells[1–3]
- Loss of MAP4K1 kinase activity alone, and in combination with checkpoint inhibition, is sufficient to enhance T cell receptor (TCR) signaling, resulting in robust anti-tumor immunity, and therefore supporting MAP4K1 as a novel and high priority target for cancer immunotherapy[1–3]
- The MAP4K family and closely related kinases in the STE20 family have been elusive drug targets despite interest in the biology of this family for cancer and autoimmune diseases[4]
- We have designed a set of potent, selective, and orally bioavailable inhibitors of MAP4K1 (BLU2069, BLU6348, and clinical candidate BLU-852)

Figure 1: MAP4K1 is a negative regulator of T cell function[1–3]



AP-1, activator protein-1; CD, cluster of differentiation; DAG, diacylglycerol; IFN-γ, interferon gamma; IL, interleukin; IP_3, inositol 1,4,5-trisphosphate; LAT, linker for activation of T cells; LCK, lymphocyte-specific protein tyrosine kinase; MAP4K1, hematopoietic progenitor kinase 1; NF-AT, nuclear factor of activated T cells; NF-kB, nuclear factor kappa B; P, phosphorylation; PLCγ, phospholipase C gamma; S_{376}, phospho-S376; SLP-76, SH2 domain-containing leukocyte protein of 76 kDa; ZAP70, zeta-chain-associated protein kinase 70.

- MAP4K1 is a SER/THR kinase selectively expressed in dendritic cells, T cells, and B cells, that acts as a negative regulator of the TCR and B cell receptor signaling, and dendritic cell maturation
- MAP4K1 knock-out or kinase dead knock-in mice exhibit enhanced tumor immunity[3,5]

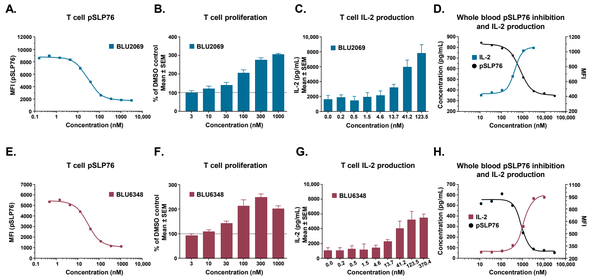
Results

Table 1: BLU2069, BLU6348, and BLU-852 are cell active subnanomolar MAP4K1 inhibitors selective against key off-targets and the kinome

	Enzyme activities IC_{50} (nM)			Cell activity IC_{50} or EC_{50} (nM)		Whole blood activity IC_{50} or EC_{50} (nM)		Selectivity
Compound	MAP4K1	LCK	MAP4K4	pSLP76[a]	IL-2[b]	pSLP76[a]	IL-2[b]	% kinome >100x
BLU2069	0.17	19	45	29	16	615	517	95
BLU6348	0.13	78	73	27	11	1033	1194	96
BLU-852	0.11	502	1196	40	11	851	1240	97

[a]IC_{50} values; [b]EC_{50} values.
IC_{50}, half-maximal inhibitory concentration; EC_{50}, half-maximal effective concentration.

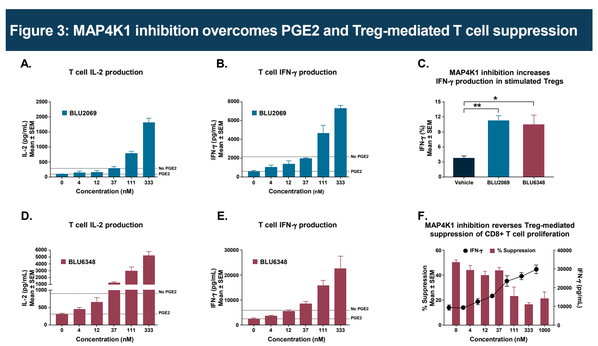
Figure 2: BLU2069 and BLU6348 inhibit TCR-stimulated pSLP76 induction and further enhance human T cell proliferation and IL-2 production



Human CD3+ T cells were stimulated with anti-CD3 and anti-CD28, and the impact of MAP4K1 inhibition with either (**top row**) BLU2069 or (**bottom row**) BLU6348 on (**A** and **E**) pSLP76 inhibition, (**B** and **F**) T cell proliferation, and (**C** and **G**) IL-2 production was measured by flow cytometry and MSD-ECL. (**D** and **H**) The impact of both compounds on pSLP76 inhibition and IL-2 production was also assessed in human whole blood by flow cytometry and MSD-ECL assay.

DMSO, dimethyl sulfoxide; MFI, median fluorescence intensity; MSD-ECL, Meso Scale Discovery-Electrochemiluminescence.

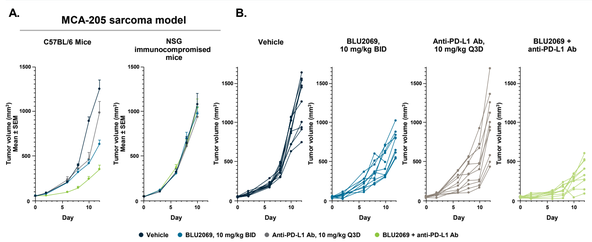
Figure 3: MAP4K1 inhibition overcomes PGE2 and Treg-mediated T cell suppression



Human CD3+ T cells were stimulated with anti-CD3 and anti-CD28 with and without the addition of 500 nM PGE2. (**A** and **D**) The ability of BLU2069 and BLU6348 to overcome PGE2-mediated suppression of IL-2 and (**B** and **E**) IFN-γ production was measured by MSD-ECL. (**C**) IFN-γ production from Tregs isolated from human whole blood and stimulated with IL-2, anti-CD3, and anti-CD28 in the presence of DMSO or 300 nM of either compound was measured by flow cytometry. (**F**) The ability of BLU6348 to reverse Treg-mediated suppression of T cell proliferation (CFSE) was assessed in a co-culture at a 1:15 ratio of Treg to effector cells. IFN-γ in the assay supernatant was also measured by MSD-ECL.

*P-value of 0.0062; **P-value of 0.0005.
CSFE, carboxyfluorescein succinimidyl ester; Treg, regulatory T cell; PGE2, prostaglandin E2.

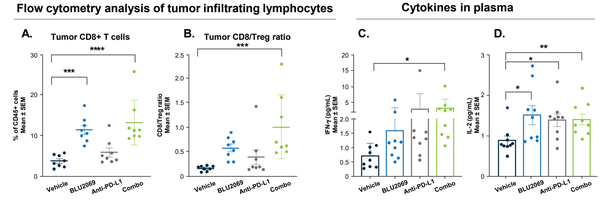
Figure 4: BLU2069 exhibits immune-dependent anti-tumor activity (MCA-205 sarcoma model)



MCA205 cells were implanted subcutaneously in the right flank of C57BL/6 or NSG mice, at 10^6 cells per mouse. Mice were subsequently randomized into treatment groups, 10 mice per group, at mean tumor volumes of 54 mm^3 for C57BL/6 and 40 mm^3 for NSG. (**A**) BLU2069 was administered by oral gavage, and anti-PD-L1 Ab (clone 10F.9G2) was administered by IP injection at the doses and schedules indicated. (**B**) Individual animal tumor volumes from the C57BL/6 experiment are shown.

BID, twice a day; IP, intraperitoneal; PD-L1 Ab, programmed death-ligand 1 antibody; NSG, NOD scid gamma; Q3D, every 3 days.

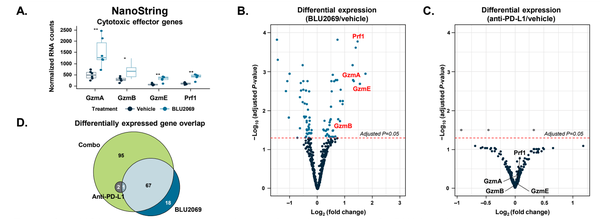
Figure 5: BLU2069 treatment increases the intratumoral CD8/Treg ratio and enhances plasma cytokine levels (MCA-205 sarcoma model)



(**A** and **B**) Tumors from MCA-205 bearing mice were collected after 5 days of dosing and the percentage of CD8 positive T cells and CD4/FOXP3 double positive Tregs was assessed by flow cytometry. (**C** and **D**) Whole blood for plasma was collected, and IFN-γ and IL-2 levels were measured by MSD-ECL.

*Adjusted P<0.05; **Adjusted P<0.01; ***Adjusted P<0.001; ****Adjusted P<0.0001.

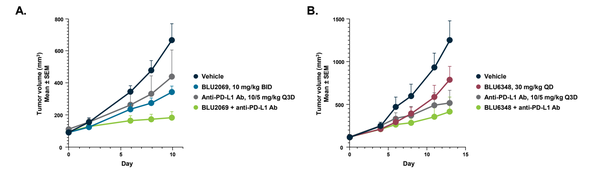
Figure 6: BLU2069 induces stronger T cell response within the tumor than anti-PD-L1 (MCA-205 sarcoma model)



Tumors from MCA-205 bearing mice were collected after 5 days of dosing for RNA isolation and were analyzed using the NanoString PanCancer IO 360™ panel. (**A**) Cytotoxic genes are upregulated in BLU2069 treated samples compared to vehicle. (**B** and **C**) Effect on transcriptome by BLU2069 compared to anti-PD-L1 treatment. (**D**) Number of differentially expressed genes overlapped under BLU2069, anti-PD-L1 and BLU2069 plus anti-PD-L1 combination treatment.

*Adjusted P<0.05; **Adjusted P<0.01.
Gzm, granzyme; Prf1, perforin 1.

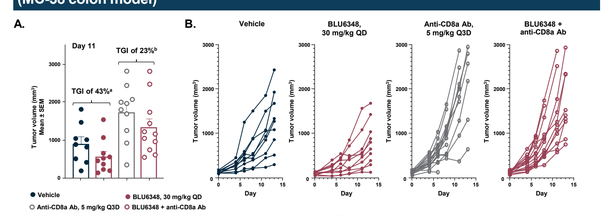
Figure 7: BLU2069 and BLU6348 exhibit monotherapy and enhanced combination anti-tumor activity (MC-38 colon model)



MC-38 cells were implanted subcutaneously in the right flank of C57BL/6 mice, at 10^6 cells per mouse. Mice were subsequently randomized into treatment groups, 10–15 mice per group, at mean tumor volumes of 100 mm^3 for the BLU2069 experiment and 118mm^3 for the BLU6348 experiment. (**A**) BLU2069 and (**B**) BLU6348 were administered by oral gavage at the doses and schedules indicated, and anti-PD-L1 Ab (provided by Roche) was administered at 10 mg/kg IV for the first dose and 5 mg/kg IP for the subsequent doses.

IV, intravenous.

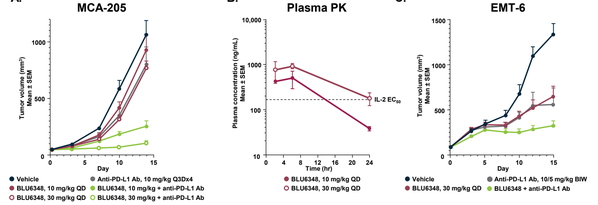
Figure 8: CD8 depletion partially reverses BLU6348-mediated efficacy (MC-38 colon model)



MC-38 cells were implanted subcutaneously in the right flank of C57BL/6 mice, at 10^6 cells per mouse. Mice were subsequently randomized into treatment groups, 10–15 mice per group, at a mean tumor volume of 124 mm^3. (**A** and **B**) BLU6348 was administered by oral gavage, and anti-CD8a Ab (clone 53-6.7) was administered by IP injection, at the doses and schedules indicated.

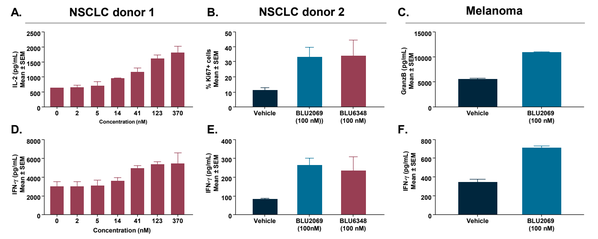
[a]Without CD8 depletion; [b]With CD8 depletion.
TGI, tumor growth inhibition.

Figure 9: BLU6348 demonstrates enhanced *in vivo* efficacy with 24-hour IL-2 EC_{50} coverage (MCA-205 sarcoma and EMT-6 breast models)



(**A**) MCA-205 and (**C**) EMT-6 cells were implanted subcutaneously in the right flank of C57BL/6 (MCA205) or BALB/c (EMT-6) mice, at 10^6 (MCA205) or $5x10^5$ (EMT-6) cells per mouse. Mice were subsequently randomized into treatment groups, 10 mice per group, at mean tumor volumes of 45 mm^3 for MCA205,and 92 mm^3 for EMT-6. BLU6348 was administered by oral gavage, and anti-PD-L1 Ab (clone 10F.9G2 for MCA205, an anti-PD-L1 Ab from Roche for EMT-6) was administered by IP injection, at the doses and schedules indicated (the Roche PD-L1 Ab administered at 10 mg/kg IV for the first dose, and 5 mg/kg IP for the subsequent doses). (**B**) Blood was collected for plasma PK analysis at the end of the BLU6348 MCA-205 efficacy study to confirm target coverage.

Figure 10: MAP4K1 inhibition enhances T cell responses in patient-derived tumor infiltrating lymphocytes



Primary human tumors from individual patients were purchased from Discovery Life Sciences. The dissociated tumors, including the infiltrating lymphocytes, were cultured in the presence of anti-CD3 and anti-CD28 (NSCLC) or anti-CD3 alone (melanoma) and the impact of compound treatment at the indicated doses on (**A, D, E, F**) cytokine production, (**B**) T cell proliferation or (**C**) Granzyme B was measured by flow cytometry and MSD-ECL.

NSCLC, non-small cell lung cancer.

Conclusions

- BLU2069 and BLU6348 are subnanomolar MAP4K1 inhibitors with an excellent selectivity profile
- Pharmacological inhibition of MAP4K1 with BLU2069 and BLU6348 supports previously reported findings in MAP4K1 knock-out and kinase-dead knock-in mice
- BLU2069 and BLU6348 enhance intratumoral immune cell activation, overcome PGE2 and Treg-mediated T cell suppression, and reduce tumor burden both as a monotherapy and in combination with checkpoint inhibition
- MAP4K1 inhibition enhanced CD3/CD28-induced cytokine and granzyme B production in patient-derived tumor infiltrating lymphocytes isolated from melanoma or NSCLC primary tumors
- This chemical matter has been further developed to deliver a clinical development candidate, BLU-852

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