

1 **ALKS 4230, a novel engineered IL-2 fusion protein with an improved cellular**  
 2 **selectivity profile for cancer immunotherapy**

3 Jared E. Lopes, Jan L. Fisher, Heather L. Flick, Chunhua Wang, Lei Sun, Marc S.  
 4 Ernstoff, Juan C. Alvarez, and Heather C. Losey

5 **Supplemental Tables**

6 **Table S1.** Cell markers used to determine potency of ALKS 4230 and rhIL-2 on mouse  
 7 splenocyte populations.

Mouse Cell Population (pSTAT5 potency assay)	Positive marker (gated on)	Negative marker
CD4 <sup>+</sup> T <sub>regs</sub>	CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>high</sup> Foxp3 <sup>+</sup>	CD11b <sup>-</sup>
NK cells	CD49b <sup>+</sup>	CD3 <sup>-</sup> CD8 <sup>-</sup>
Memory phenotype CD8 <sup>+</sup> T cells	CD3 <sup>+</sup> CD8 <sup>+</sup> CD44 <sup>+</sup>	CD11b <sup>-</sup>

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9 **Table S2.** Cell markers used to determine potency of ALKS 4230 and rhIL-2 on human  
 10 lymphocyte populations.

HUMAN CELL POPULATION	GATED BIOMARKERS
CD4 <sup>+</sup> T <sub>regs</sub>	CD14 <sup>-</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>high</sup> FOXP3 <sup>+</sup>
NK cells	CD14 <sup>-</sup> CD3 <sup>-</sup> CD56 <sup>+</sup>
Naive CD8 T cells	CD14 <sup>-</sup> CD3 <sup>+</sup> CD8 <sup>+</sup> CD28 <sup>+</sup> CD45RA <sup>+</sup> CD95 <sup>-</sup>
Central/Transitional memory CD8 T cells	CD14 <sup>-</sup> CD3 <sup>+</sup> CD8 <sup>+</sup> CD28 <sup>+</sup> CD45RA <sup>-</sup> CD95 <sup>+</sup>
Effector memory CD8 T cells	CD14 <sup>-</sup> CD3 <sup>+</sup> CD8 <sup>+</sup> CD28 <sup>-</sup> CD45RA <sup>-</sup> CD95 <sup>+</sup>
Terminal effector CD8 T cells	CD14 <sup>-</sup> CD3 <sup>+</sup> CD8 <sup>+</sup> CD28 <sup>-</sup> CD45RA <sup>+</sup> CD95 <sup>+</sup>

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12 **Table S3.** Cell markers used in human *ex vivo* expansion experiments.

Cell Population	Markers
T <sub>regs</sub>	CD14 <sup>-</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>+</sup> CD127 <sup>low/-</sup> ICOS <sup>+/-</sup>
Central memory CD8 <sup>+</sup> T cells	CD3 <sup>+</sup> CD8 <sup>+</sup> CD95 <sup>+</sup> CD28 <sup>+</sup> CCR7 <sup>+</sup> CD45RA <sup>-</sup>
Central memory CD4 <sup>+</sup> T cells	CD3 <sup>+</sup> CD4 <sup>+</sup> CD95 <sup>+</sup> CD28 <sup>+</sup> CCR7 <sup>+</sup> CD45RA <sup>-</sup>

CD56 <sup>+</sup> NK cells	CD14 <sup>-</sup> CD3 <sup>-</sup> CD56 <sup>+</sup>
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14 **Table S4.** Cell Markers Used in C57BL/6 Mouse PD Biomarker.

Mouse Cell Population	Positive marker (gated on)	Negative marker
CD4 <sup>+</sup> T <sub>regs</sub>	CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>+</sup> Foxp3 <sup>+</sup>	CD11b <sup>-</sup> CD127 <sup>-/low</sup>
Conventional CD4 <sup>+</sup> T cells	CD3 <sup>+</sup> CD4 <sup>+</sup>	CD11b <sup>-</sup> Foxp3 <sup>-</sup>
NK cells	NK1.1 <sup>+</sup> CD122 <sup>+</sup>	CD3 <sup>-</sup>
Mature NK cells	NK1.1 <sup>+</sup> CD11b <sup>+</sup> CD27 <sup>+</sup> CD122 <sup>+</sup>	CD3 <sup>-</sup>
Cytolytic NK cells	NK1.1 <sup>+</sup> CD11b <sup>+</sup> CD122 <sup>+</sup>	CD3 <sup>-</sup> CD27 <sup>-</sup>
Memory-phenotype CD8 <sup>+</sup> T cells	CD3 <sup>+</sup> CD8 <sup>+</sup> CD44 <sup>+</sup> CD122 <sup>+</sup>	CD11b <sup>-</sup>

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16 **Table S5.** Protein sequences utilized in design of ALKS 4230

Molecule	Primary Sequence
Recombinant human IL-2 (aldesleukin)	APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFYMPKKATELKH LQCLEEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT
Human IL-2R $\alpha$ extracellular domain	ELCDDDPPEIPHATFKAMAYKEGTM LNCECKRGFRRIKSGSLYMLCTGNSSHSSWD NQCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPWEN EATERIYHFVVGQMVVYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTG
ALKS 4230	SKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFSQSIIS TLTGGSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFYMPKKATEL HLQCLEEEELKPLEEVLNLAQSGGGSELCDDDPPEIPHATFKAMAYKEGTM LNCEC KRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATRNTTKQVTPQPEEQKERKT EMQSPMQPVDQASLPGHCREPPWENEATERIYHFVVGQMVVYQCVQGYRALHRGP AESVCKMTHGKTRWTQPQLICTG

17 Green = sequence encompassing helices A and B of IL-2

18 Aqua = sequence encompassing helices C and D of IL-2

19 Gray = sequence derived from IL-2R $\alpha$ 

20 Yellow = linker sequence

21 Red = C125S

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23 **Table S6.** Data collection and refinement statistics for ALKS 4230.  
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Data Collection	
Beamline	Advanced Light Source, 4.2.2
Wavelength (Å)	1.000
Temperature (K)	100
Resolution (Å)	3.4
Total reflections	81980 (8357)
Unique reflections	7538 (750)
Redundancy	10.9 (11.1)
Completeness (%)	100 (100)
$I/\sigma$	12.8 (1.1)
$R_{\text{merge}}$	0.185

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Refinement	
Resolution (Å)	63.6 - 3.4
No. reflections used in refinement	7516 (750)
Reflections used in $R_{\text{free}}$	421 (51)
$R_{\text{free}} / R_{\text{cryst}}$	0.297 / 0.275
B-factors (Å <sup>2</sup> )	172.0
Atoms / residues	2057 / 258
Ramachandran	87.2 % most favored, 11.6 % allowed, 1.2 % outliers
R.m.s deviations bond lengths (Å) / angles (°)	0.002 / 0.654
TLS groups	8
Resolution (Å)	63.6 - 3.4
PDB ID	6VWU

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27 Values in parentheses are for the outer shell (3.522 - 3.4 Å)

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32 **Table S7.** Pharmacokinetic properties in mice.

Test Article	Dosing Route	Dose	C <sub>max</sub>	T <sub>max</sub>	AUC <sub>0-24h</sub>	AUC <sub>0-∞</sub>	t <sub>1/2</sub>	MRT <sub>0-24h</sub>	%F
		(µg)	(nM)	(h)	(nM•h)	(nM•h)	(h)	(h)	
ALKS 4230	IV	3	54.2	0.083	140	155	8.0	5.6	
	SC	8	7.65	4	119	183	15	9.6	47
rhIL-2	SC	20	38.2	0.25	60.9	61.0	3.3	1.2	
	SC	50	124	0.5	137	138	3.6	1.1	

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