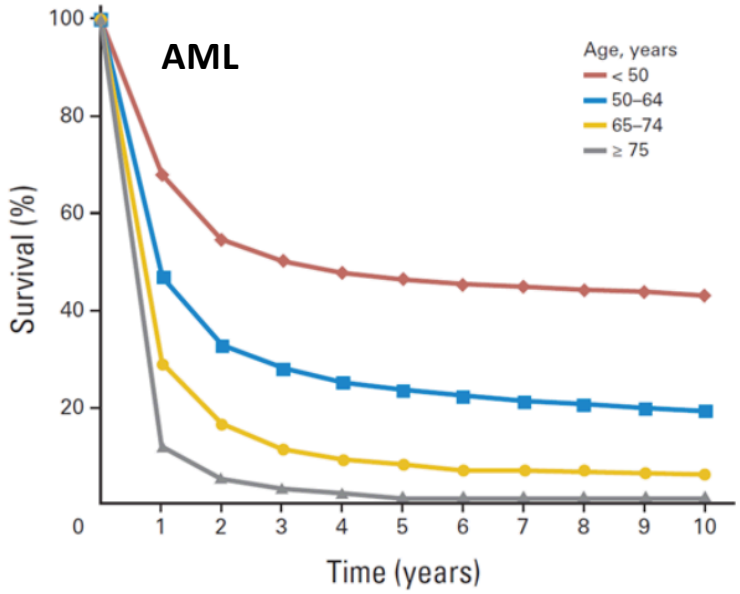
AXL: the New Kid on the Innate Immune Checkpoint Block

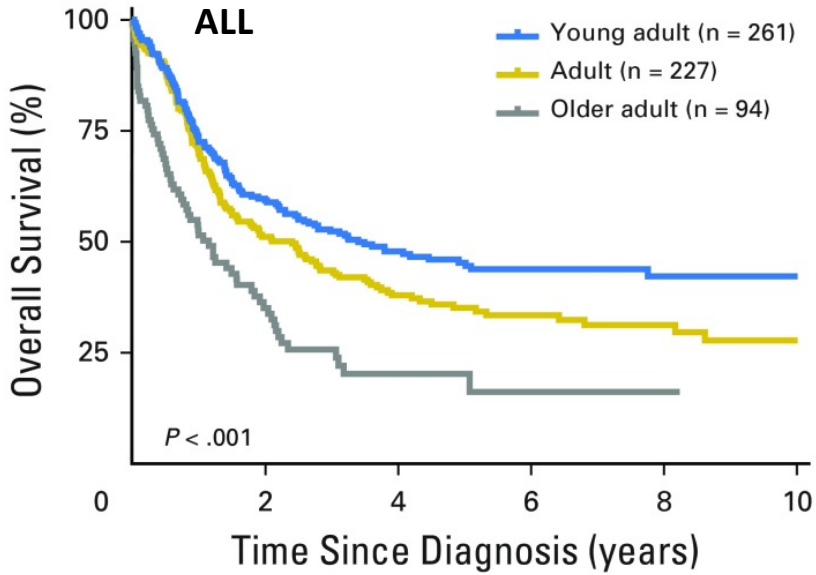
Hind Medyouf, Ph.D.

Institute for Tumor Biology and Experimental Therapy
Frankfurt am Main, Germany
Medyouf@gsh.uni-Frankfurt.de

Acute Leukemia

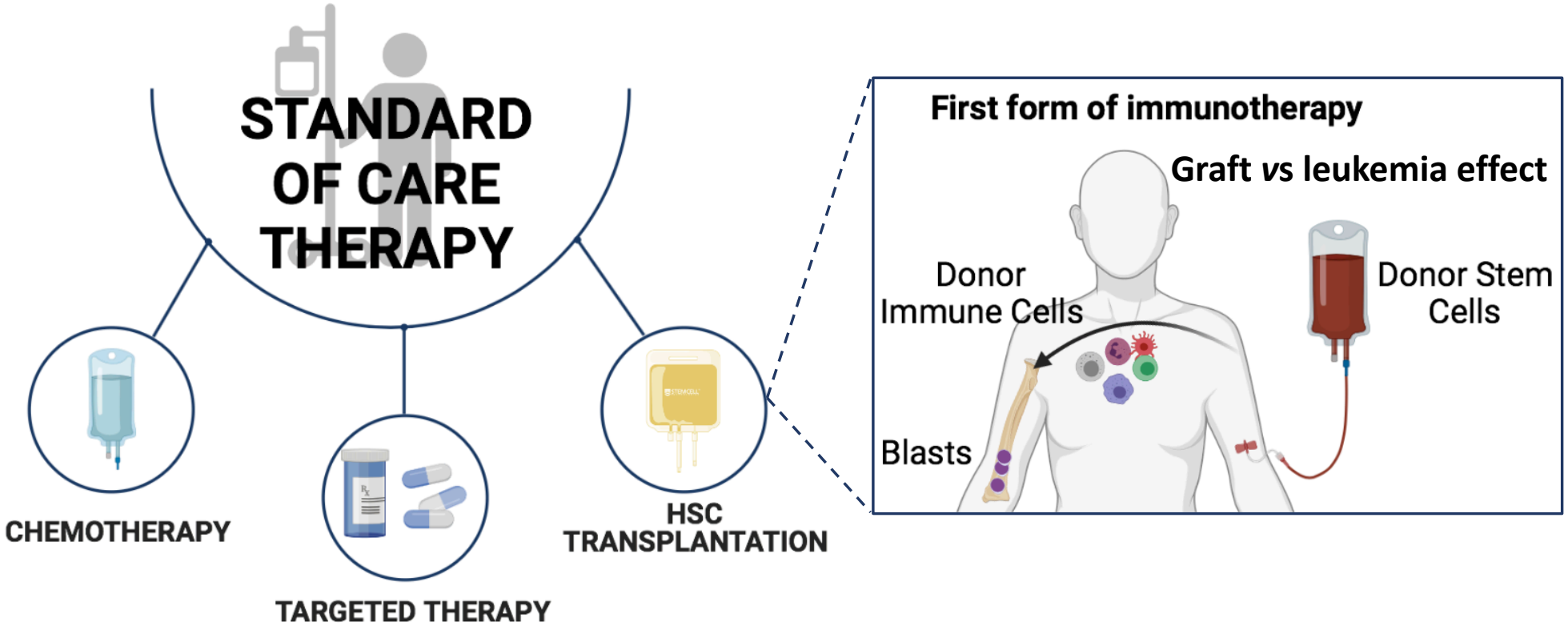
- Devastating blood cancers
- Particularly deadly in adults and elderly





Klepin et al. 2014, Roberts et al, 2017

Leukemia Therapeutic Arsenal



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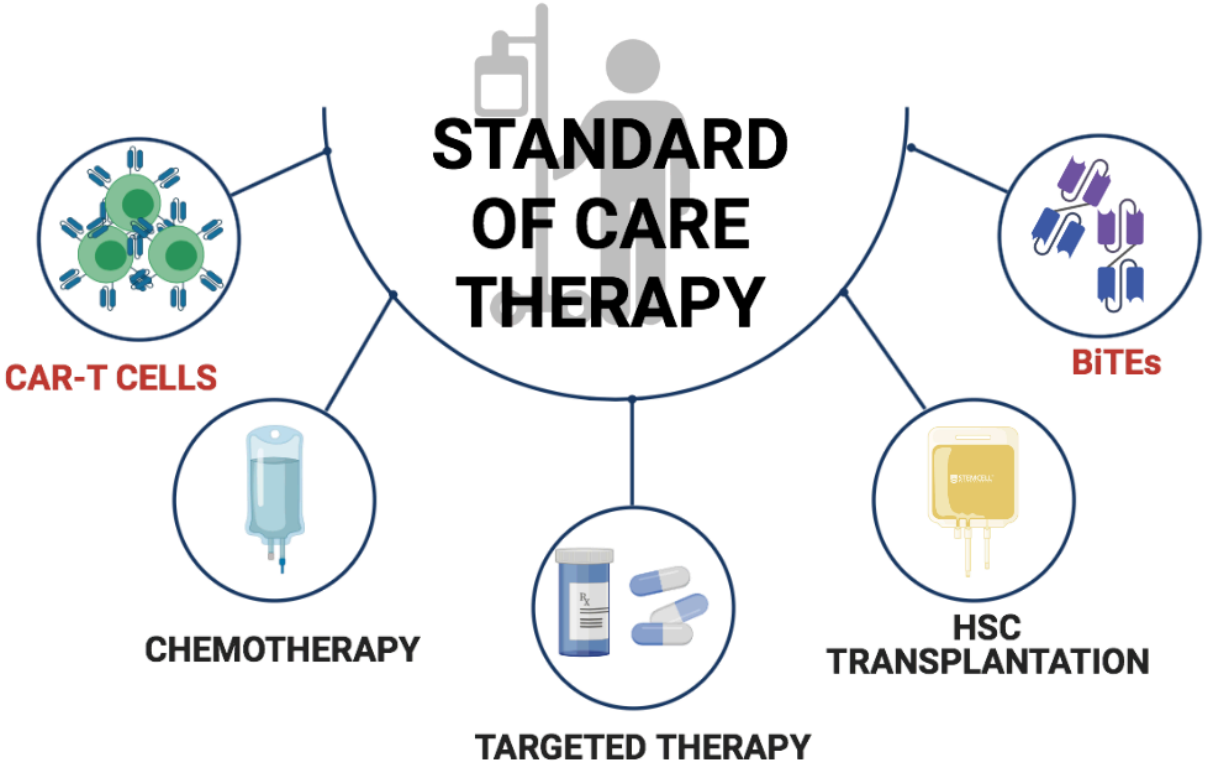
Leukemia Therapeutic Arsenal



- Require tumor specific target
- Efficacy can be limited by an immune-suppressive TME

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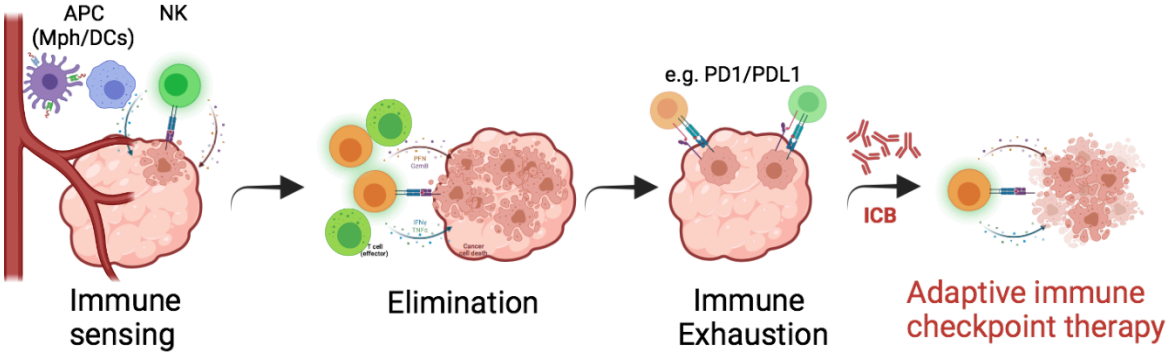
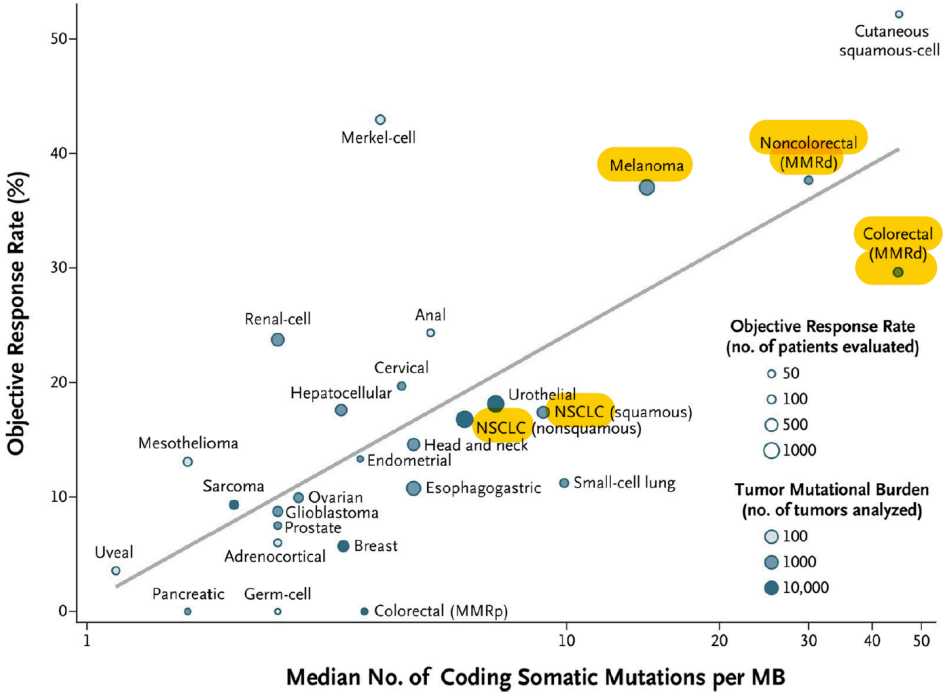
Adaptive Immune Checkpoint Blockade





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Arora S, Adv Ther, 2019

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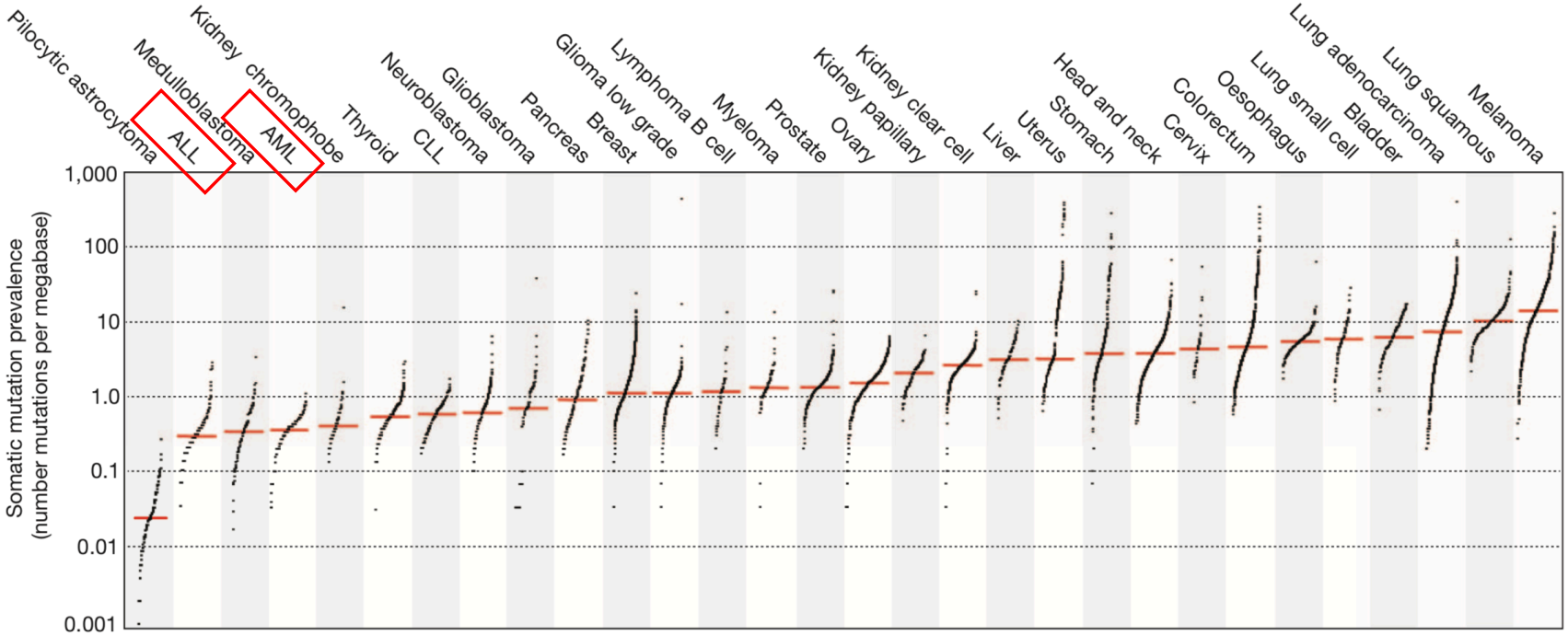
Prevalence of Mutations in Solid vs Blood Cancers



- **Acute leukemia = Low somatic mutations**
  - **Reduced immune recognition**
- **Unattractive candidates for adaptive immune checkpoint blockade**

Alexandrov, Nature, 2013

Hypothesis & Study Aims

Hypothesis: Leukemia evade immune control by actively dampening innate immunity

- Cellular and molecular mechanisms involved?
  - Potential for clinical exploitation?

GAS6 & AXL are Associated with Poor Outcome in AML

Leukemia. 2014 June ; 28(6): 1252–1258. doi:10.1038/leu.2013.371.

GAS6 expression identifies high-risk adult AML patients: potential implications for therapy

Susan P. Whitman[1], Jessica Kohlschmidt[1,2], Kati Maharry[1,2], Stefano Volinia[3], Krzysztof Mrózek[1], Deedra Nicolet[1,2], Sebastian Schwind[1], Heiko Becker[1], Klaus H. Metzeler[1], Jason H. Mendler[1], Ann-Kathrin Eisfeld[1], Andrew J. Carroll[4], Bayard L. Powell[5], Thomas H. Carter[6], Maria R. Baer[7], Jonathan E. Kolitz[8], Il-Kyoo Park[1], Richard M. Stone[9], Michael A. Caligiuri[#1], Guido Marcucci[#1], and Clara D. Bloomfield[#1]

Regular Article

MYELOID NEOPLASIA

Axl, a prognostic and therapeutic target in acute myeloid leukemia mediates paracrine crosstalk of leukemia cells with bone marrow stroma

Isabel Ben-Batalla,[1,2] Alexander Schultze,[1,2] Mark Wroblewski,[1,2] Robert Erdmann,[1,2] Michael Heuser,[3] Jonas S. Waizenegger,[1,2] Kristoffer Riecken,[4] Mascha Binder,[1] Denis Schewe,[5] Stefanie Sawall,[1,2] Victoria Witzke,[1,2] Miguel Cubas-Cordova,[1,2] Melanie Janning,[1,2] Jasmin Wellbrock,[1] Boris Fehse,[4] Christian Hagel,[6] Jürgen Krauter,[3] Arnold Ganser,[3] James B. Lorens,[7] Walter Fiedler,[1] Peter Carmeliet,[8,9] Klaus Pantel,[2] Carsten Bokemeyer,[1] and Sonja Loges[1,2]

Leukemia (2015) **29,** 2382–2389

www.nature.com/leu

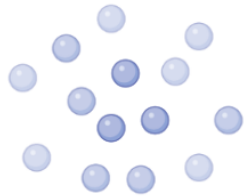
ORIGINAL ARTICLE

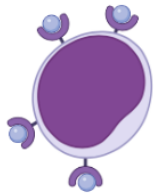
Receptor tyrosine kinase Axl is required for resistance of leukemic cells to FLT3-targeted therapy in acute myeloid leukemia

I-K Park[1], B Mundy-Bosse[1], SP Whitman[1], X Zhang[2], SL Warner[3], DJ Bearss[3], W Blum[1,4], G Marcucci[1,4] and MA Caligiuri[1,4]

Tumor intrinsic
Pro-oncogenic function

GAS6

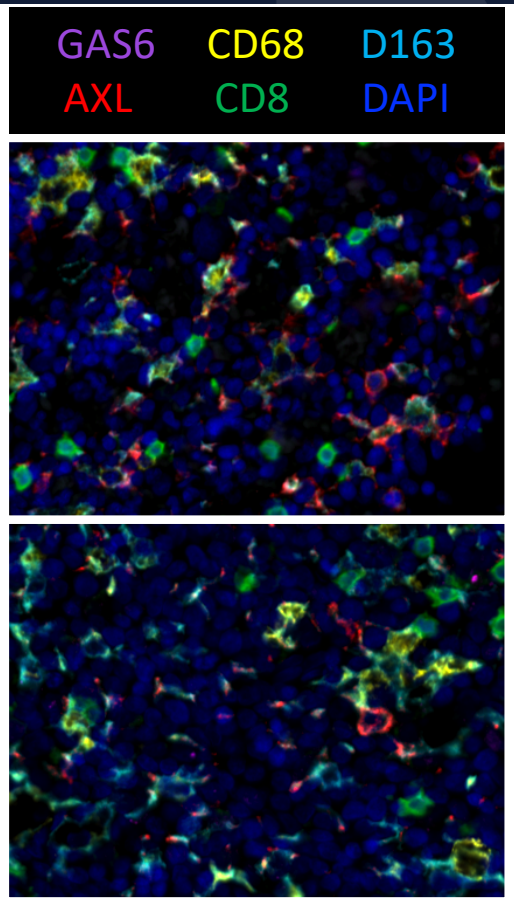




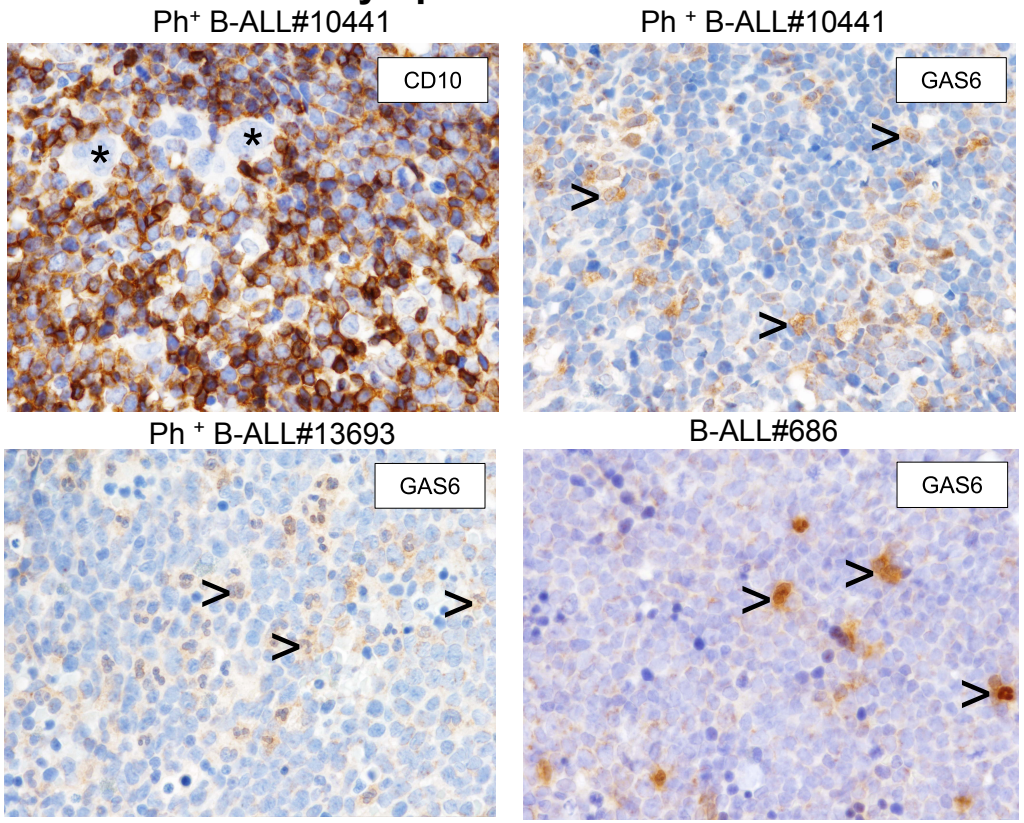
AXL+
Tumor Cells

Leukemia-associated Macrophages Express High Levels of GAS6 and AXL

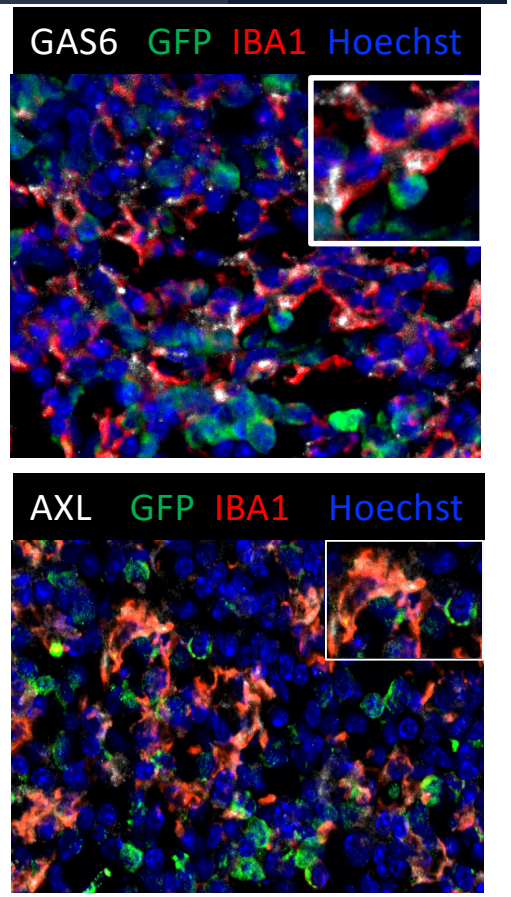
Human



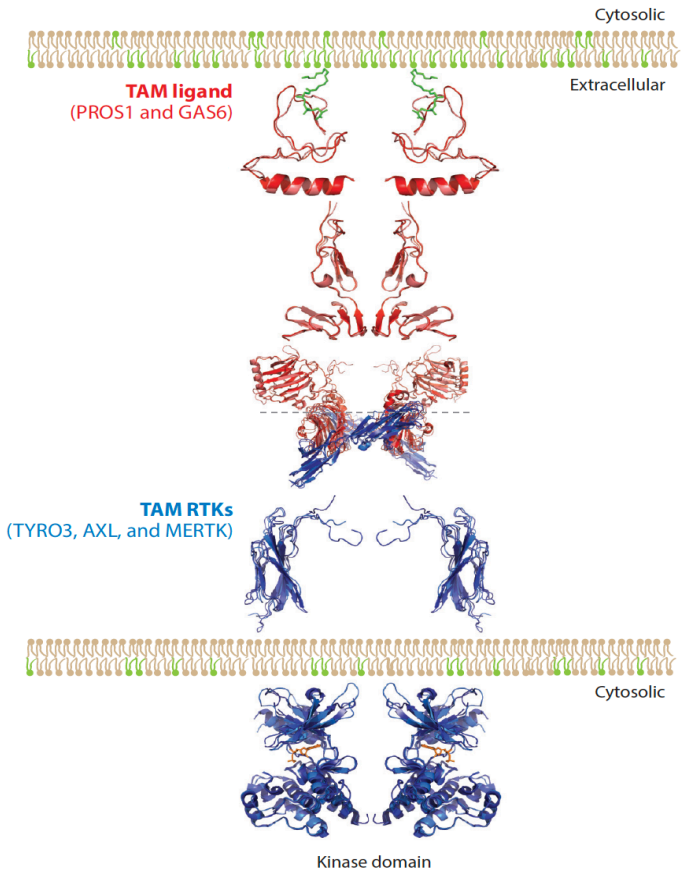
Acute Lymphoblastic Leukemia



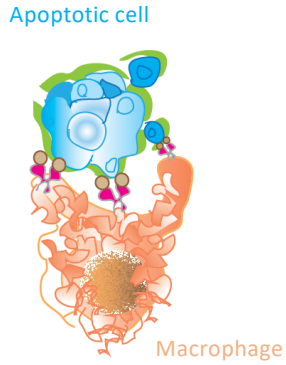
Mouse



TAM (TYRO3, AXL, MERTK) Receptors and Ligands, GAS6 & PROS1

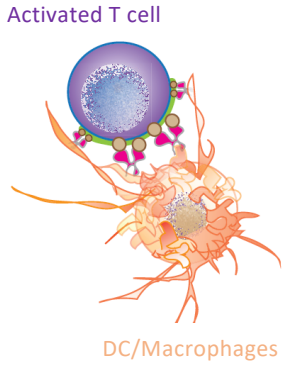


Efferocytosis



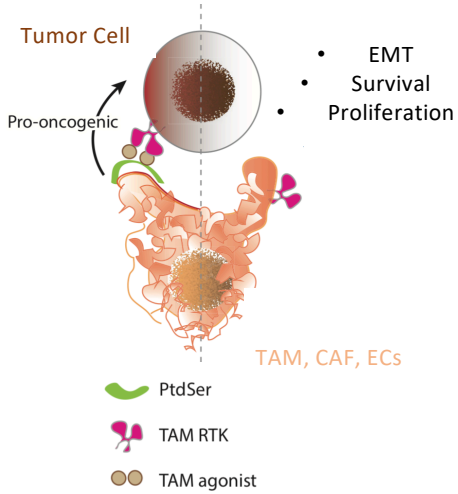
Anti-inflammatory/
Tissue repair
(M2-Like)

Infection



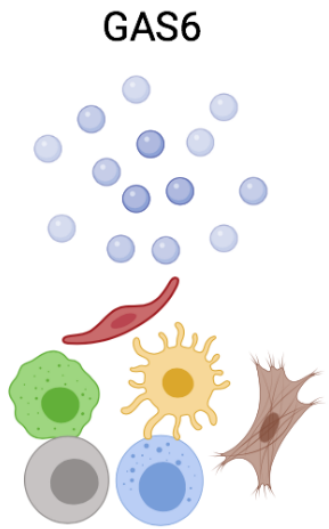
Dampening
Inflammation

Pro-oncogenic



Adapted from Rothlin et al, Annual Rev Immunol. 2015

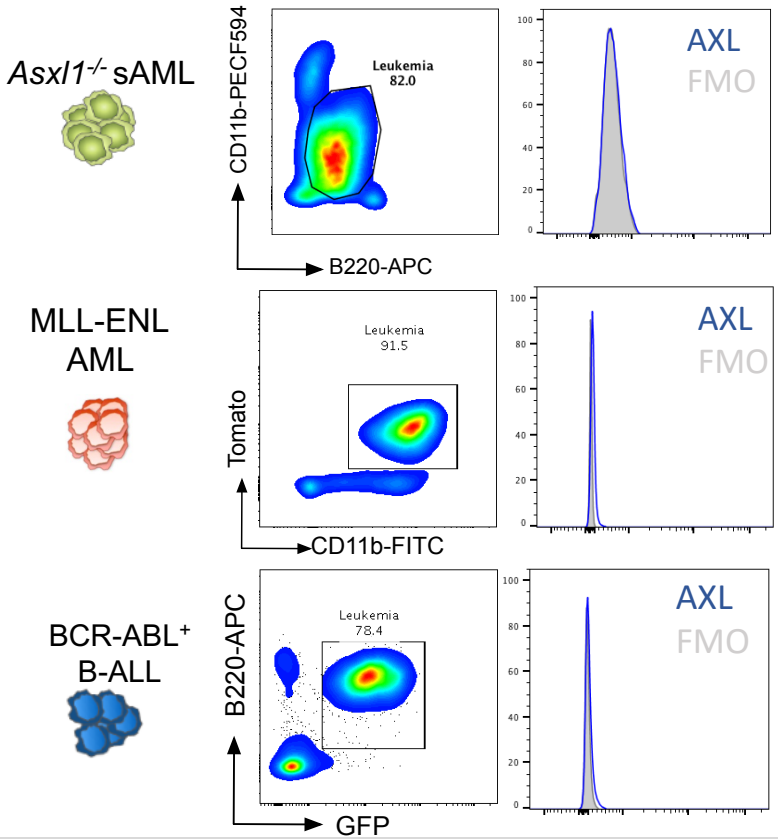
Tumor extrinsic function of GAS6/AXL axis in leukemia?



AXL^{+} stromal cells
(Immune cells: DC, Mph, NK)

Experimental Models & Approach

AXL-negative leukemia models

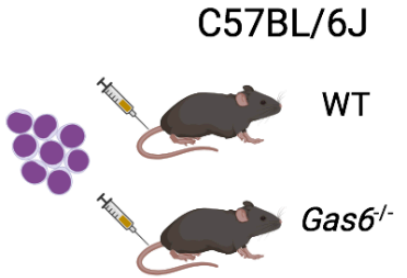


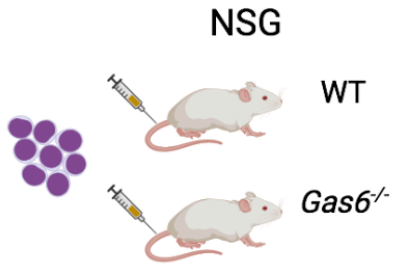
- **Leukemia transplantation in *Gas6*$^{-/-}$ recipients**

Immune competent | **Immune deficient**

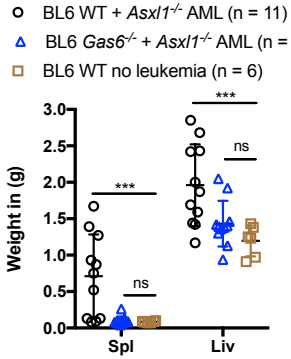
Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

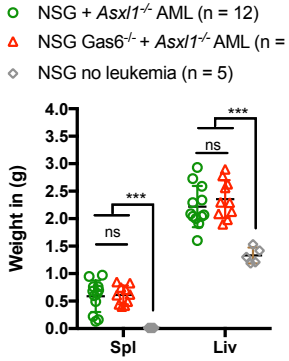
GAS6 Ablation Hampers Leukemic Growth by Promoting Anti-Leukemic Immunity



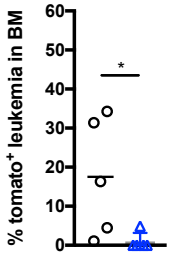


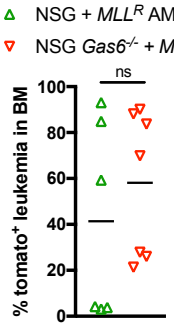
Asxl1$^{-/-}$ AML



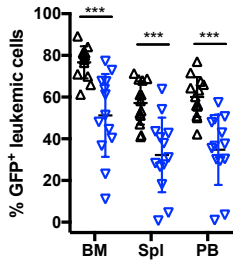


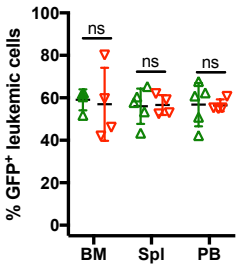
MLL-ENL AML





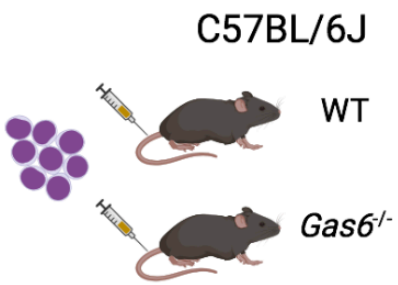
BCR-ABL1+ B-ALL

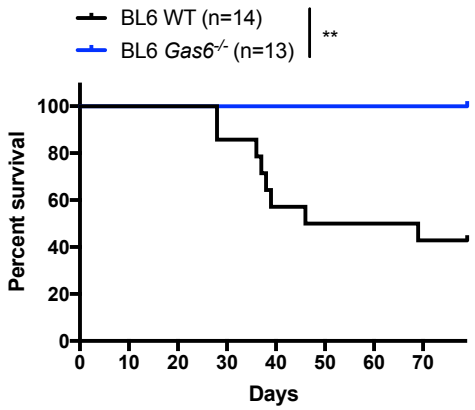


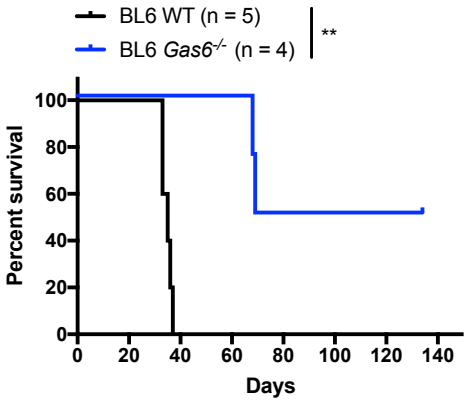


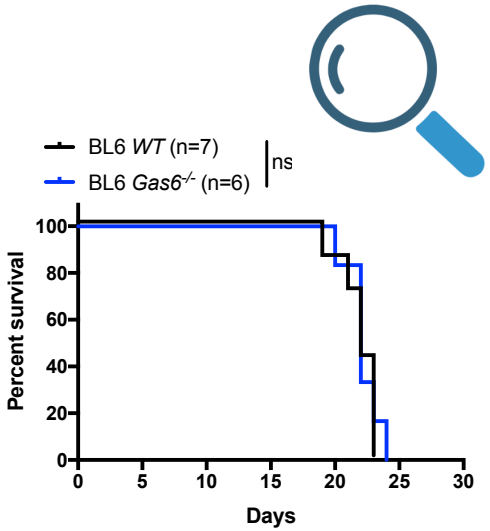
Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

GAS6 Ablation Hampers Leukemic Growth by Promoting Anti-Leukemic Immunity



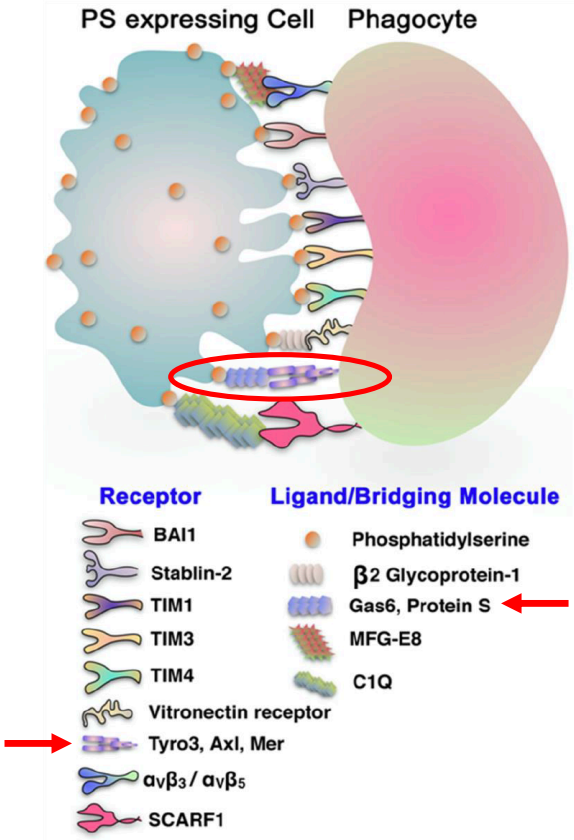


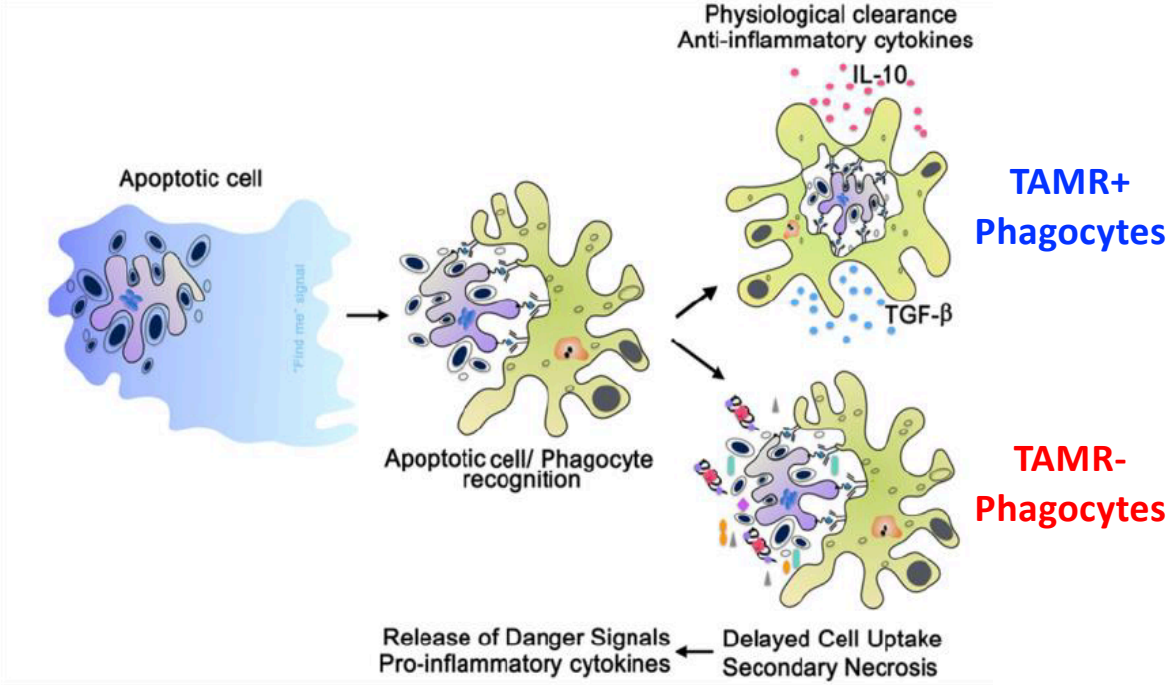




Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

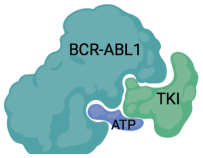
Apoptotic Cells Limit Innate Immunity



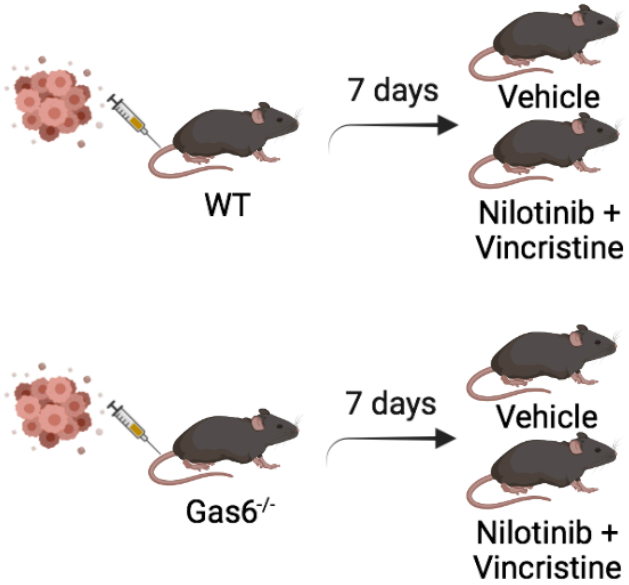


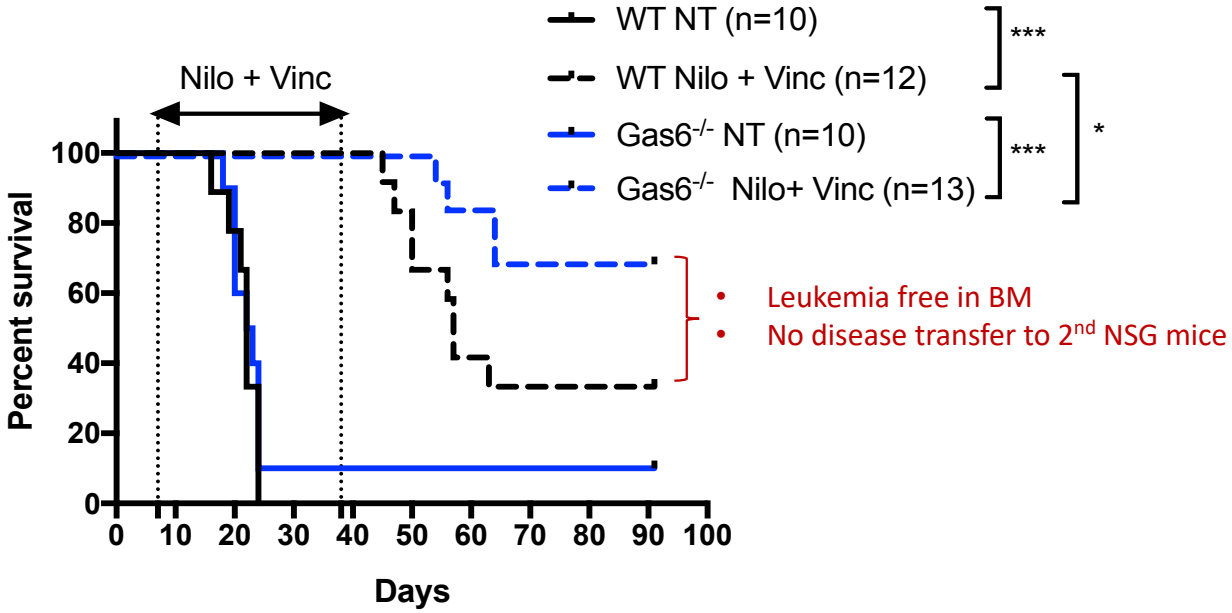
GAS6 Deficiency Synergizes with Standard of Care Therapy to Eradicate Leukemia

Targeted Therapy: Nilotinib



Chemotherapy: Vincristine

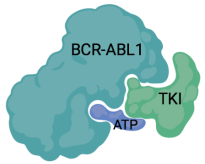


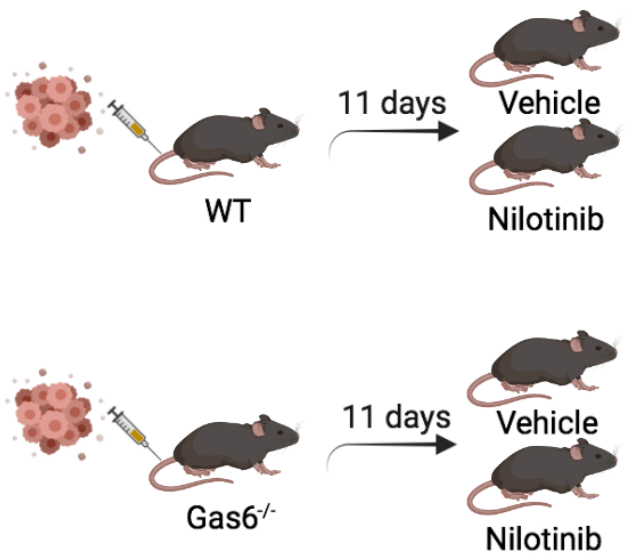


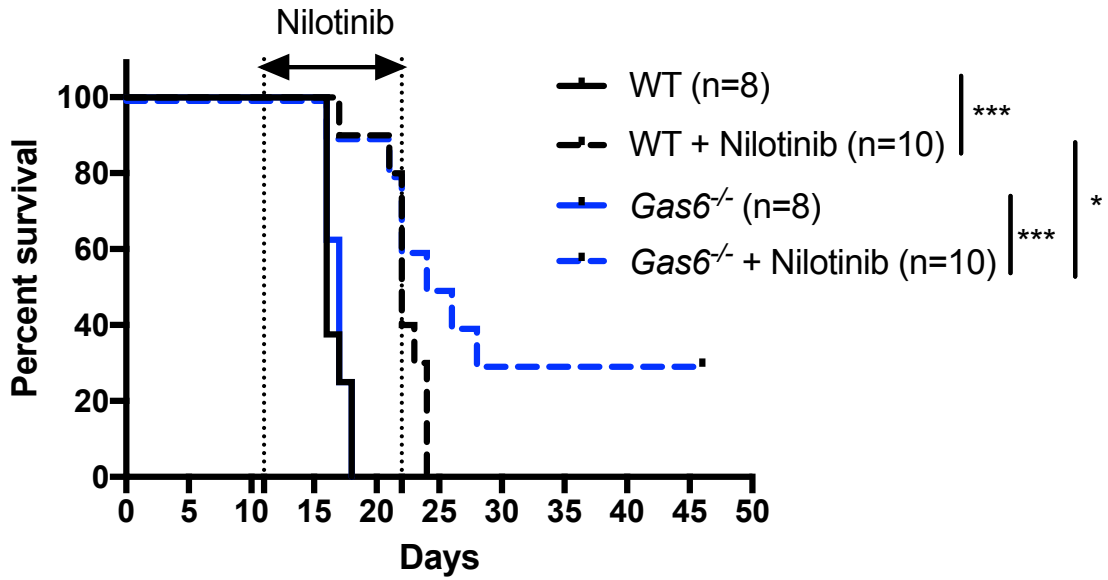
Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

GAS6 Deficiency Synergizes with Reduced Intensity Regimen to Eradicate Leukemia

Targeted Therapy: Nilotinib



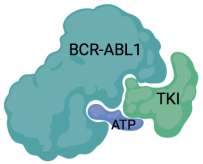


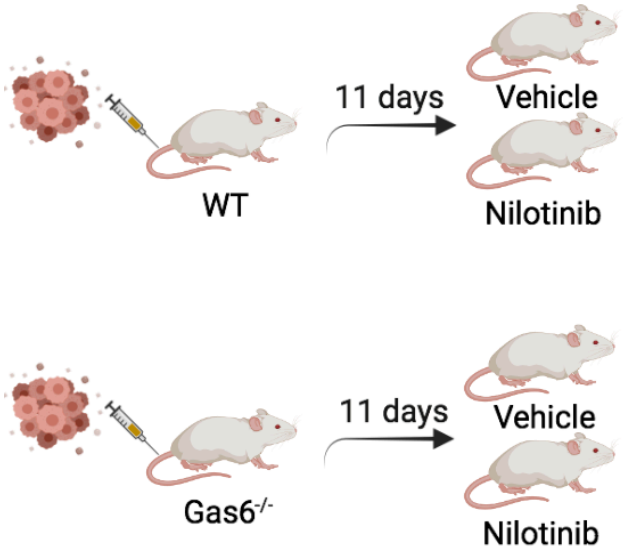


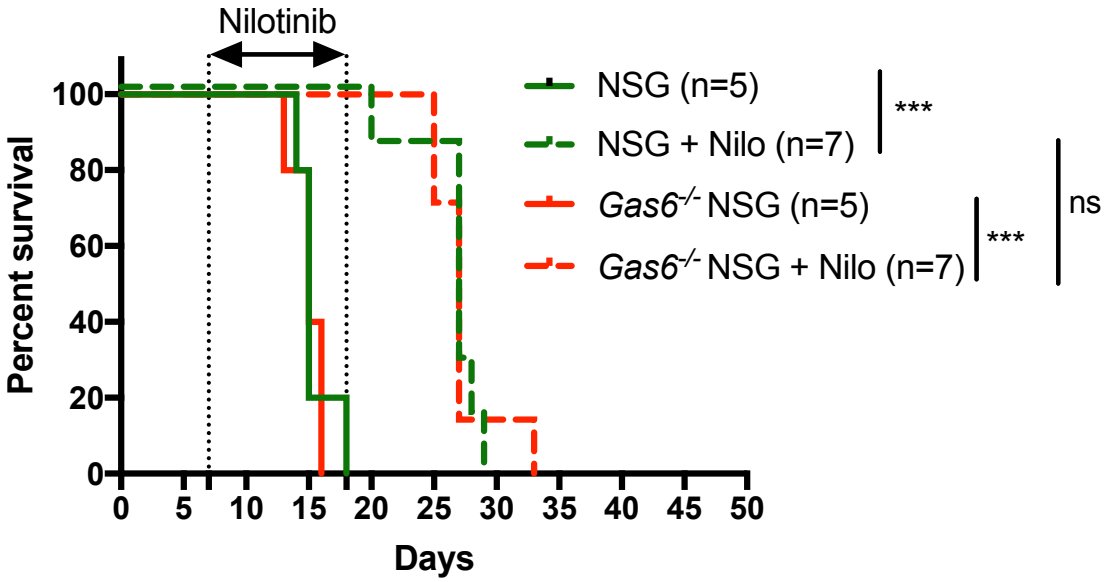
Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

…and these Effects are Dependent on a Functional Immune System!

Targeted Therapy: Nilotinib



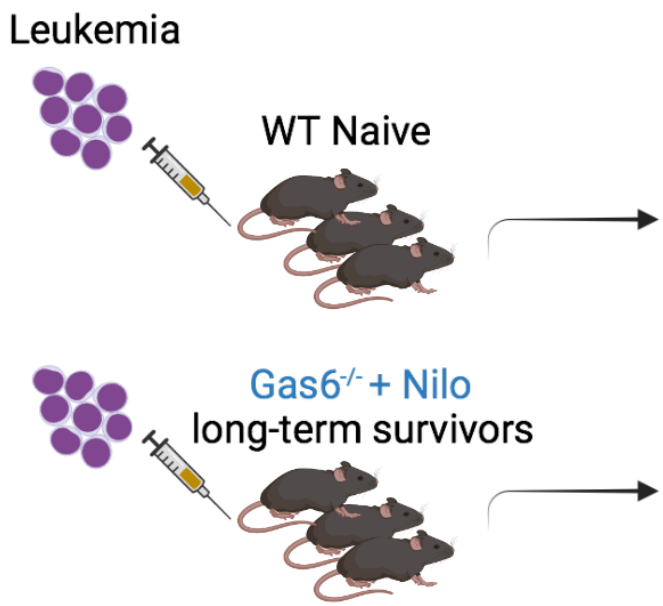


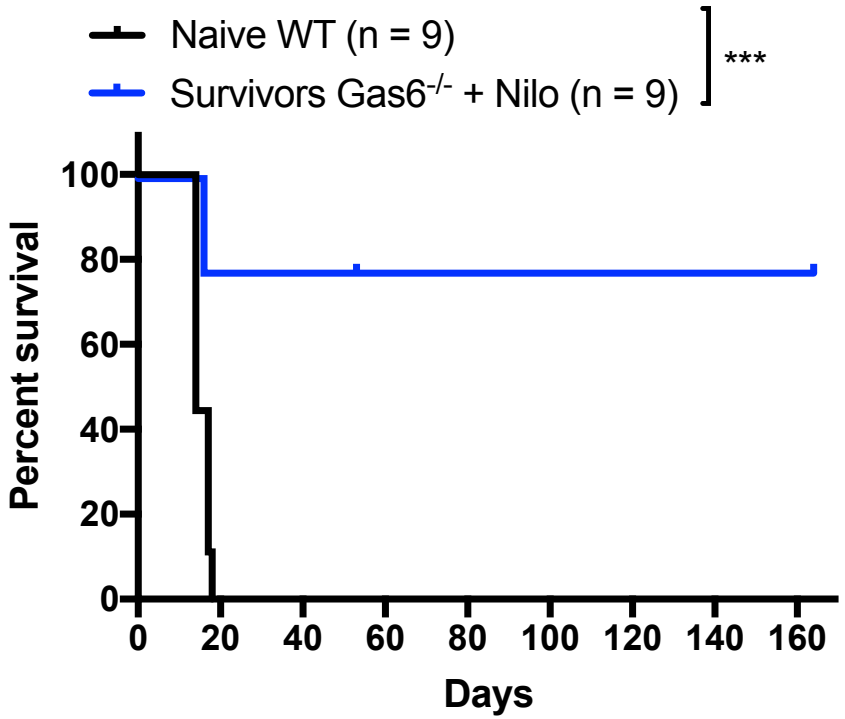


Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

Acquisition of Anti-leukemic Immune Memory in TKI-exposed GAS6$^{-/-}$ Animals

Re-challenge



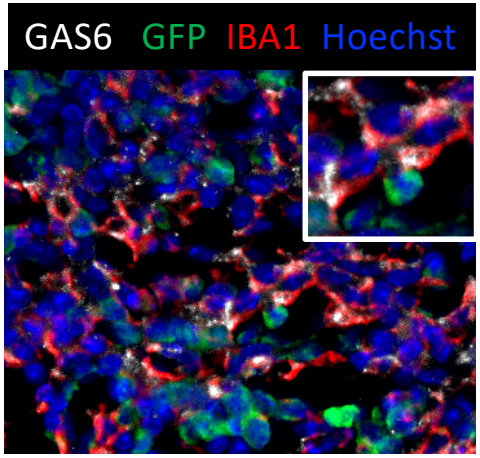


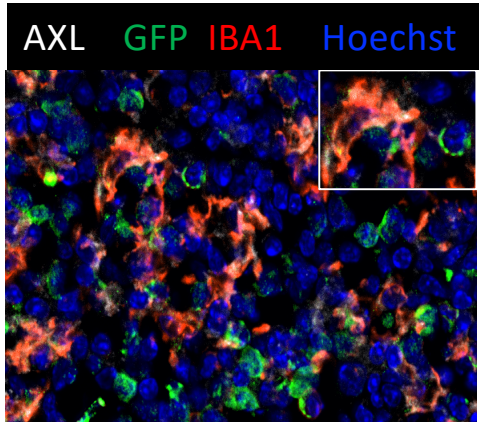
Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

Cellular Mediator(s) of GAS6 Effects?

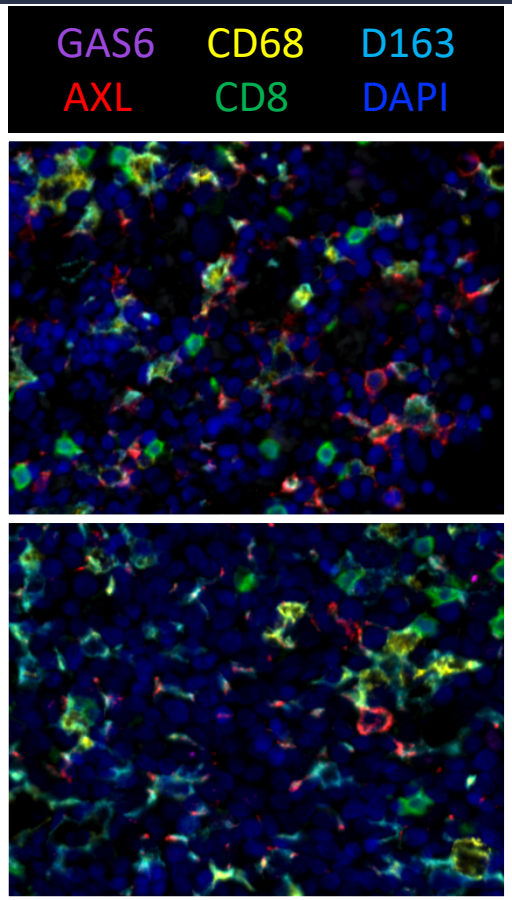
Leukemia-associated Macrophages Express High Levels of GAS6 and AXL

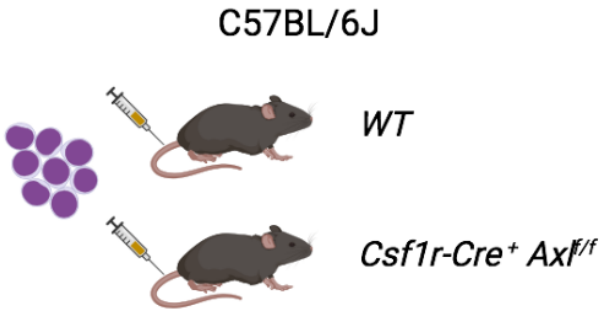
Mouse





Human





Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

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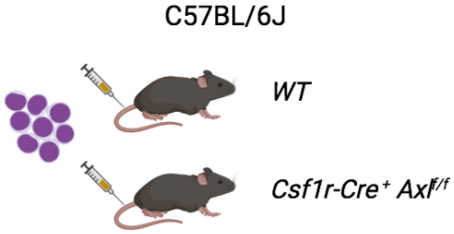
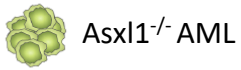
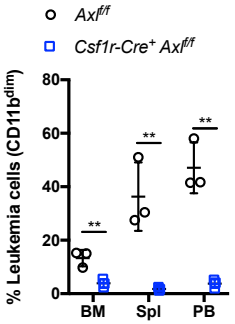
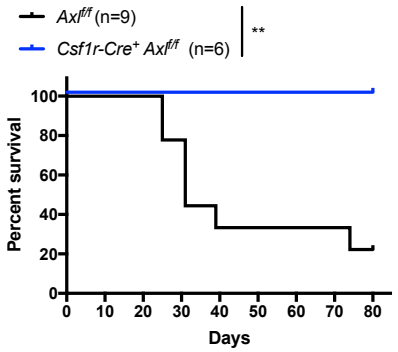
AXL-ablation in Mononuclear Phagocytes Hampers Leukemic Growth





Asxl1$^{-/-}$ AML





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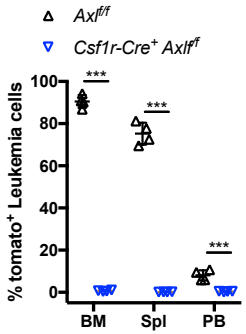
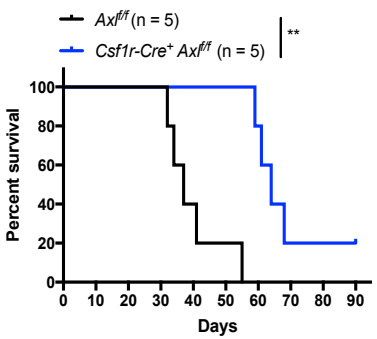
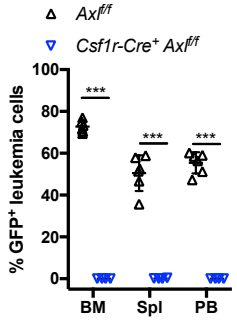
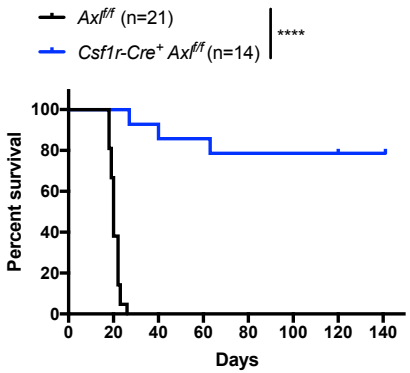




BCR-ABL1+ B-ALL



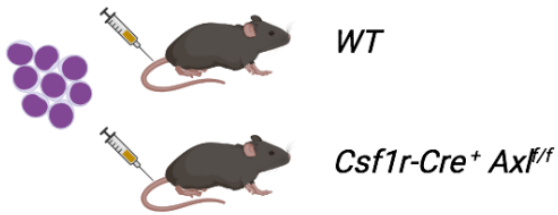


- Protective effects were lost when macrophages depleted
- Protective effects recapitulated in *Csf1r-Cre Socs3$^{f/f}$* mice

Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

AXL-ablation in Macrophages not DCs Triggers Anti-leukemic Immunity

C57BL/6J



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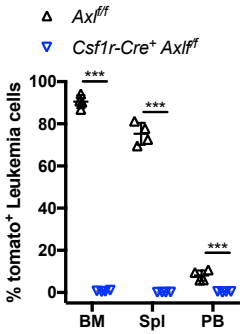

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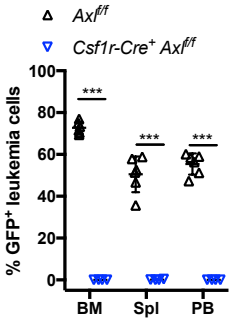
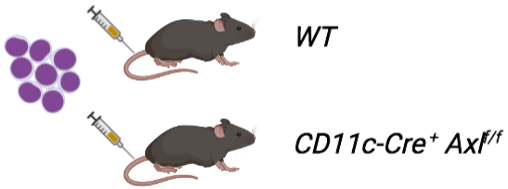
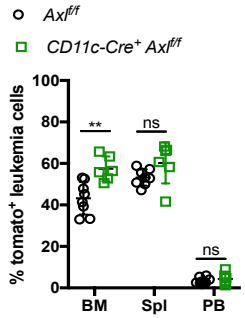
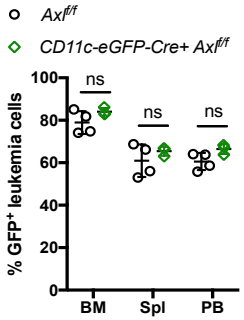


C57BL/6J





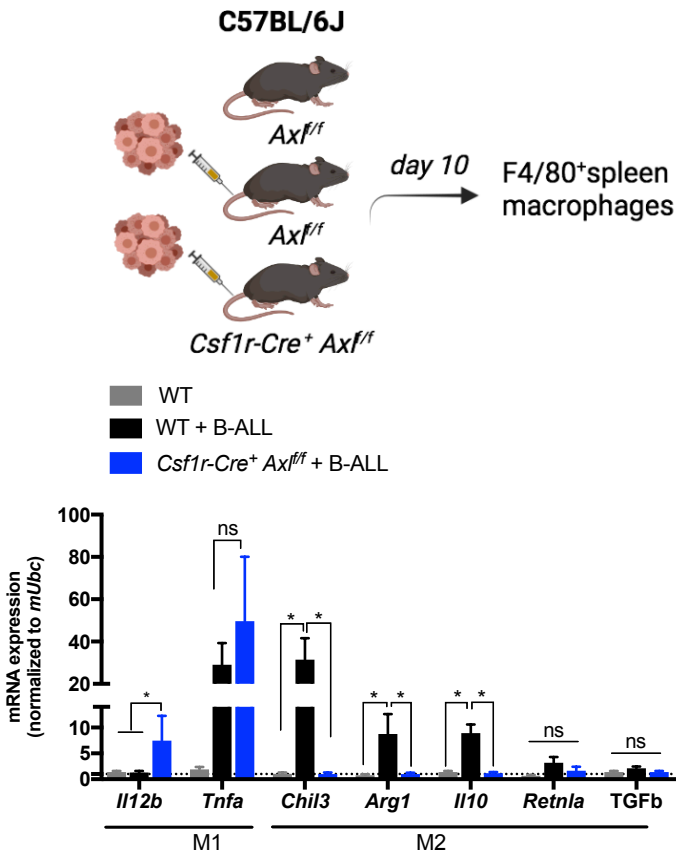


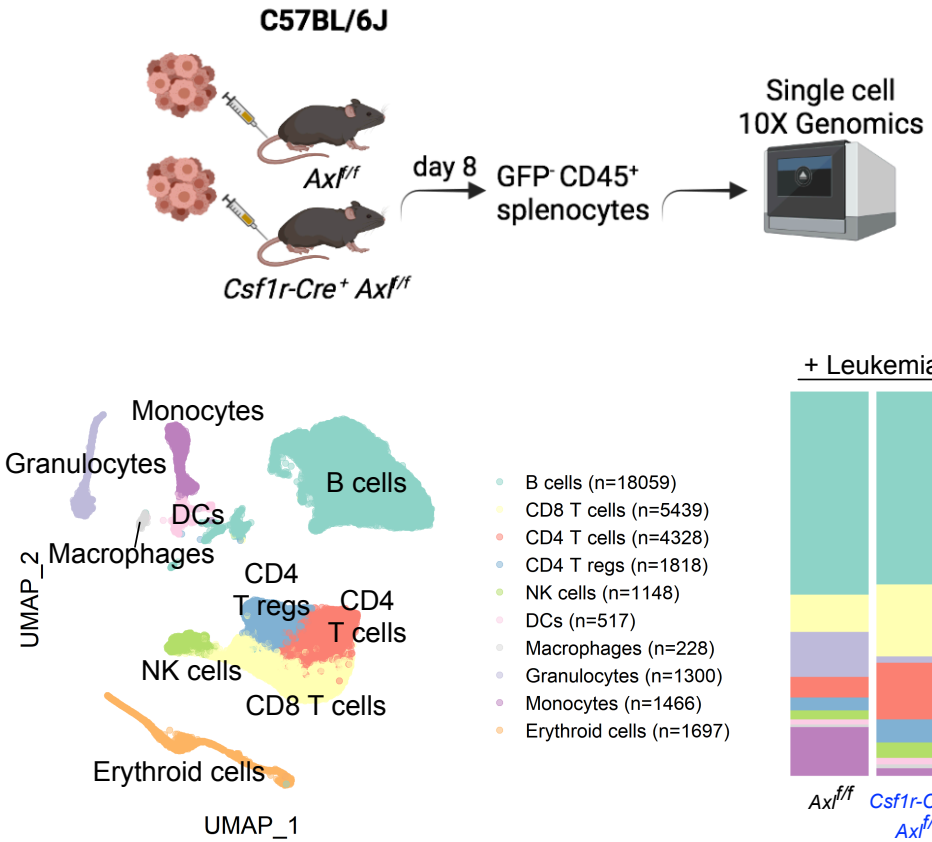
Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

Downstream Effector Cells?

AXL-ablation Rewires Macrophages Towards an Inflammatory Fate

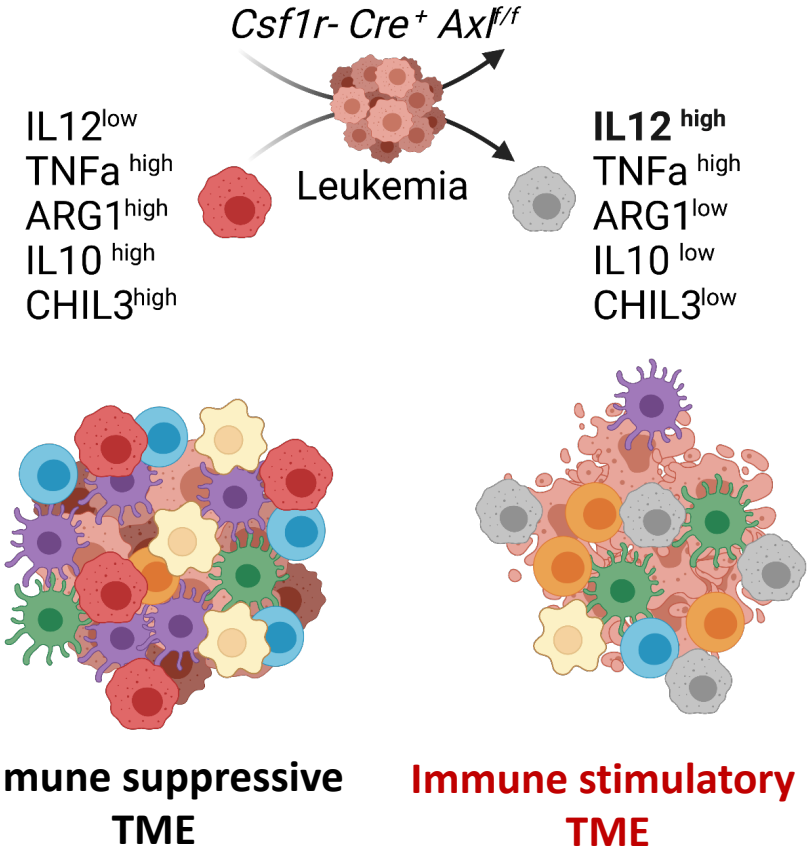
B-ALL





Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

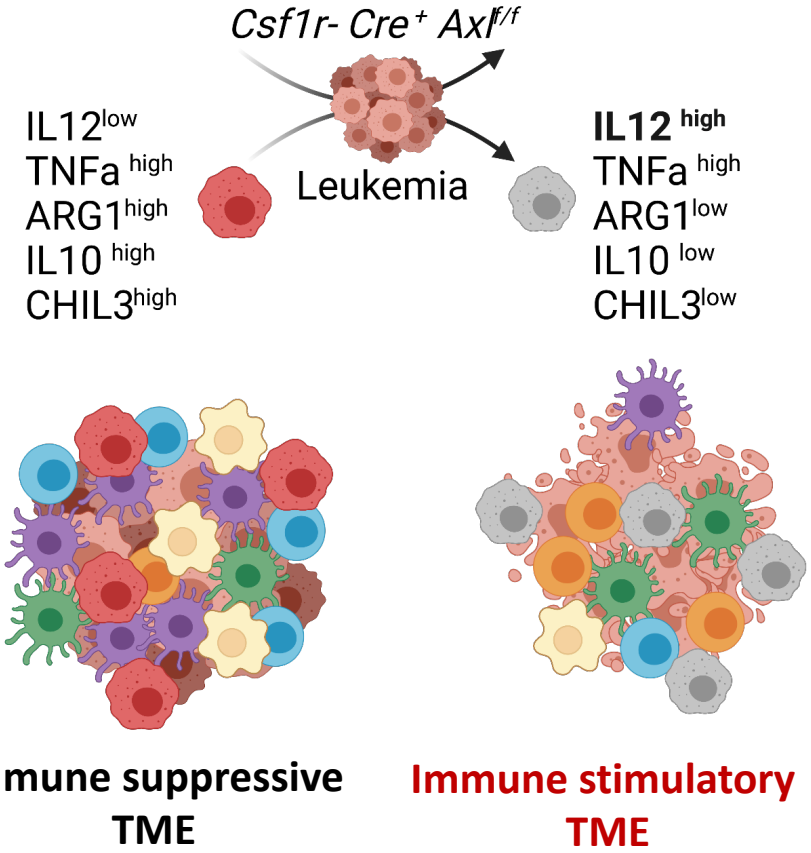
…Leading to a Major Switch in the Tumor Immune Microenvironment



	$Axl^{f/f}$	$Csf1r\text{-}Cre^{+}\ Axl^{f/f}$
Anti-Inflamm. Mph	+++	+
Pro-Inflamm. Mph	+	+++
$CD11b^{+}$ cDC2	+++	+
$CD8^{+}$ cDC1	++	++
$Ly6C^{high}$ MDSCs	+++	+
Ratio CD8/CD4 T cells	+	+++
$Foxp3^{+}$ $CD4^{+}$ T regs	+++	+
NK cells	+	+++

Generated with Biorender.com

…Leading to a Major Switch in the Tumor Immune Microenvironment



	Axl$^{f/f}$	*Csf1r- Cre*$^{+}$ *Axl*$^{f/f}$
Anti-Inflamm. Mph	+++	+
Pro-Inflamm. Mph	+	+++
CD11b^{+} cDC2	+++	+
CD8^{+} cDC1	++	++
Ly6C^{high} MDSCs	+++	+
Ratio CD8/CD4 T cells	+	+++
Foxp3^{+} CD4^{+} T regs	+++	+
NK cells	+	+++

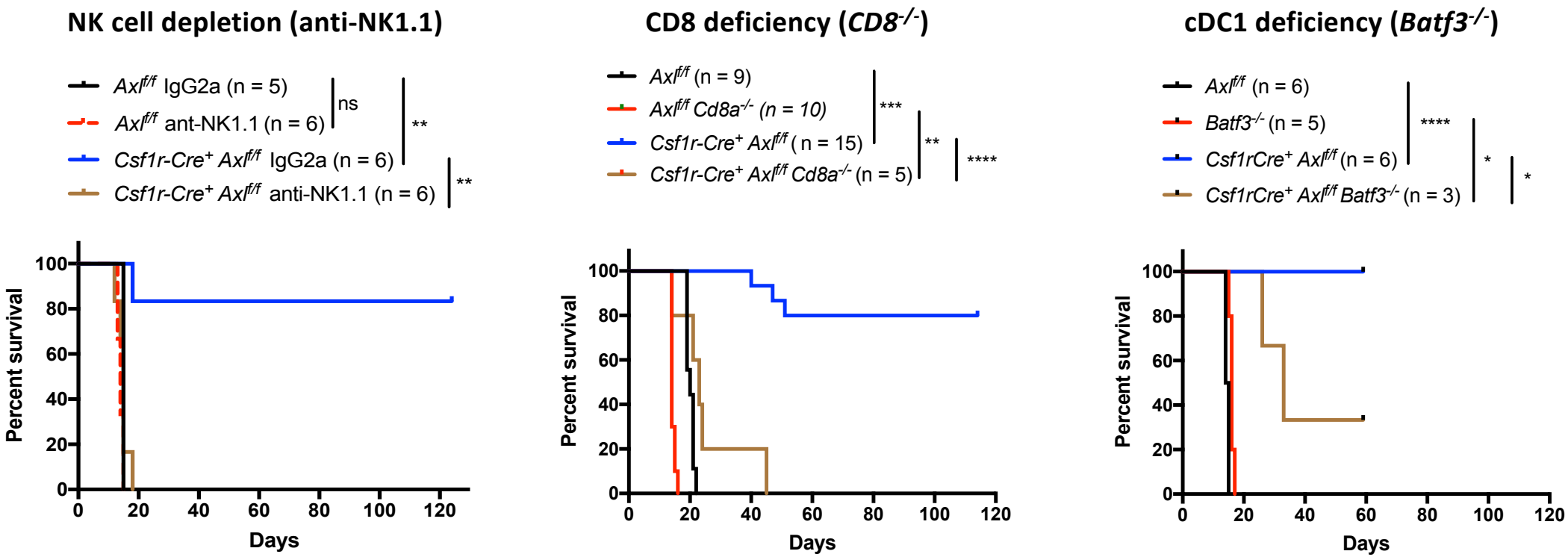
Generated with Biorender.com

Correlation *vs* Causation

Credit to Oded Rechavi

AXL-deficient Macrophages Effectively Engage Effector Lymphocytes

B-ALL



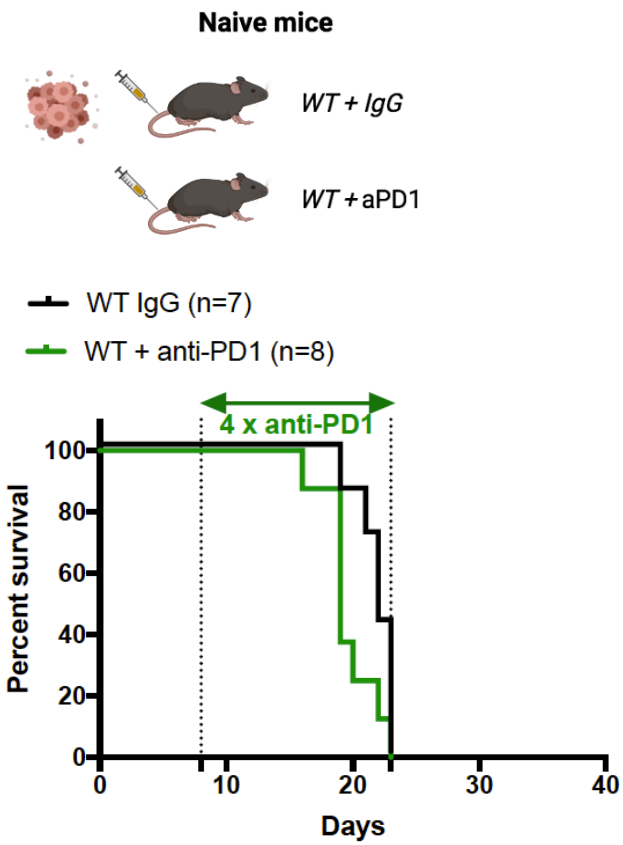
Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

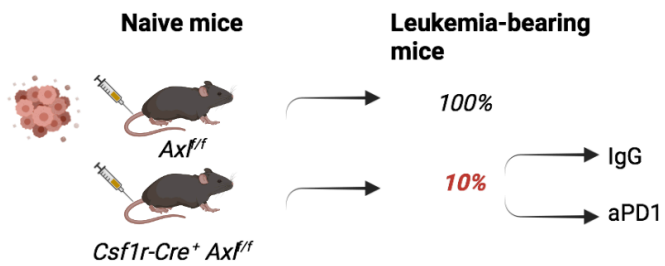
GEORG SPEYER HAUS
INSTITUTE FOR TUMOR BIOLOGY
AND EXPERIMENTAL THERAPY

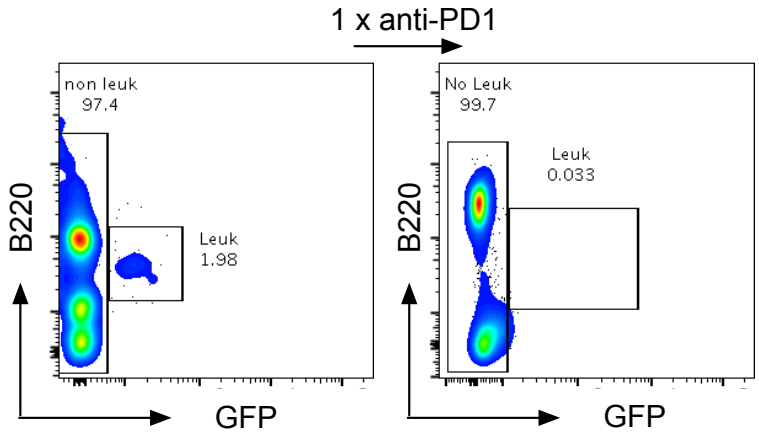
Can AXL Ablation Elicit Adaptive Checkpoint Therapy?

AXL Blockade Elicits Checkpoint Therapy

BCR-ABL1+ B-ALL



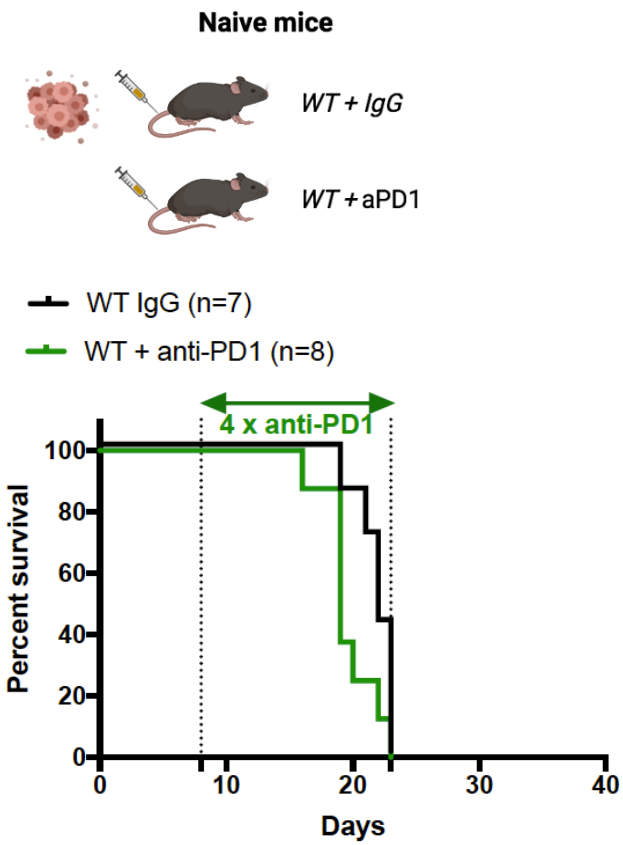


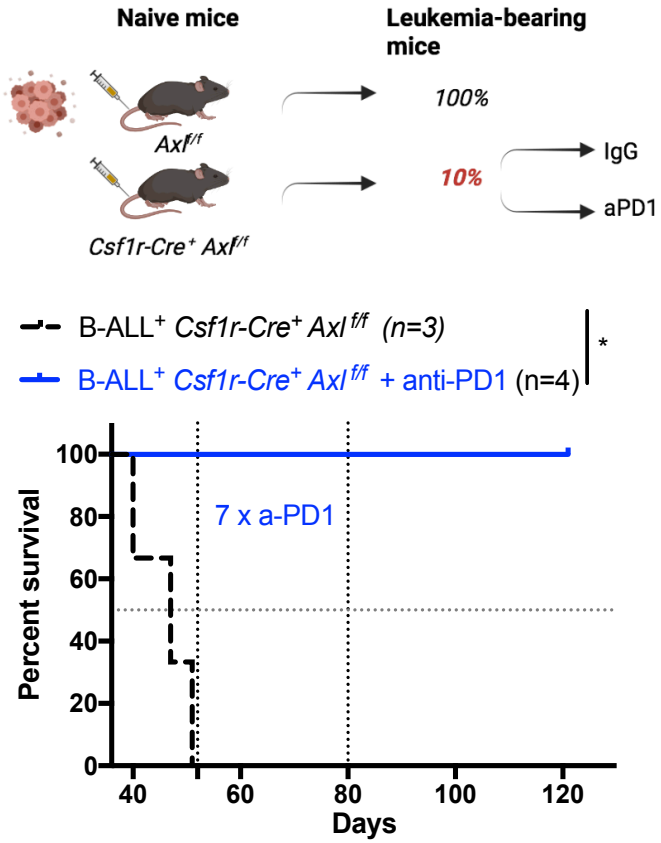


Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

AXL Blockade Elicits Checkpoint Therapy

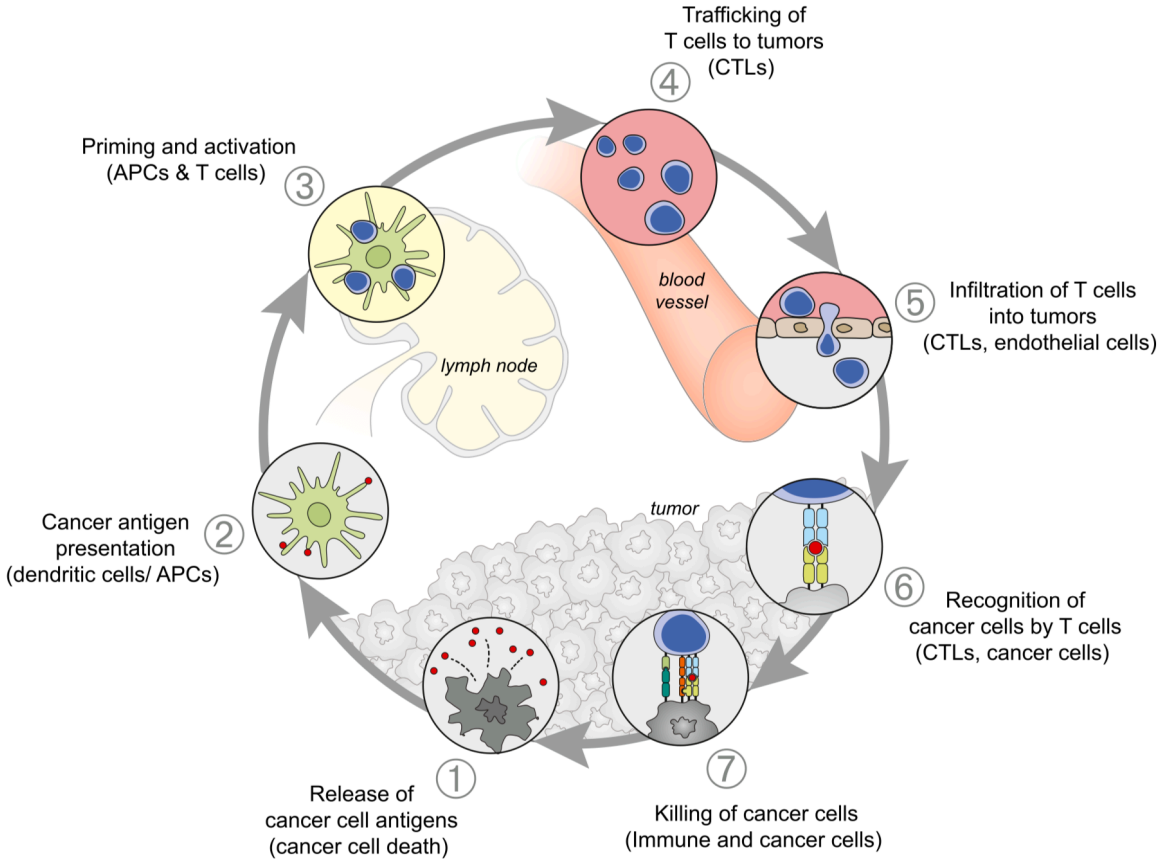
BCR-ABL1+ B-ALL





Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

AXL-deficient Macrophages Kick-start the "Cancer-Immunity Cycle" & Elicit Adaptive Checkpoint Blockade

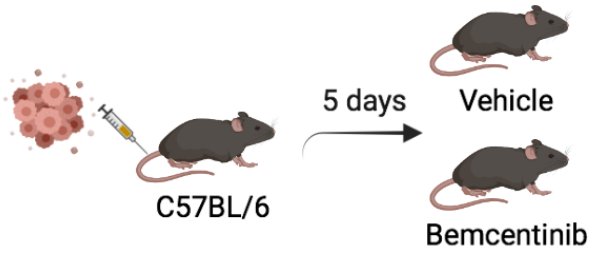


Chen and Mellman, Immunity, 2013

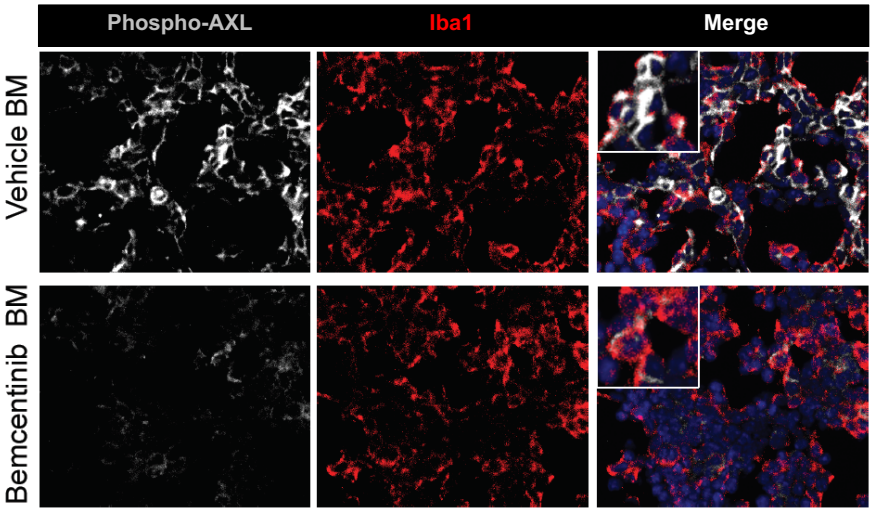
Clinical Translation?

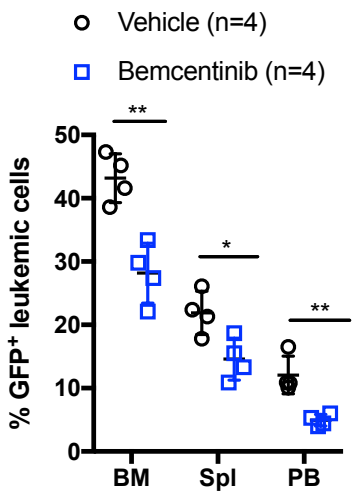
A Clinical Grade AXL Inhibitor Triggers Anti-Leukemic Immunity

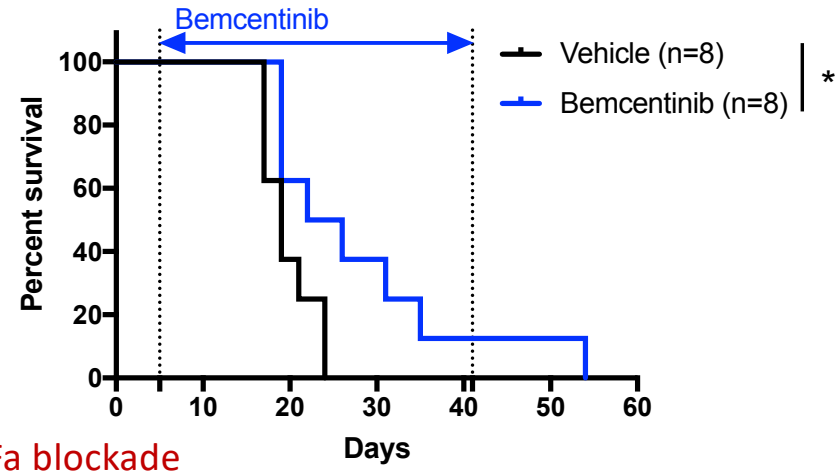
B-ALL



- Target inhibition
- Early endpoint burden
- Survival benefit





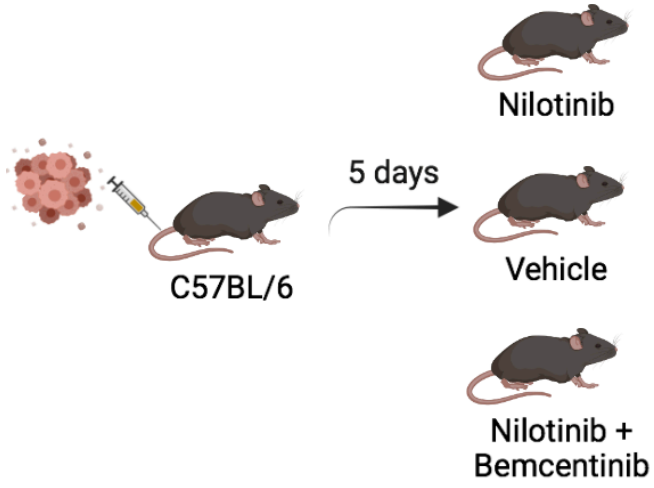


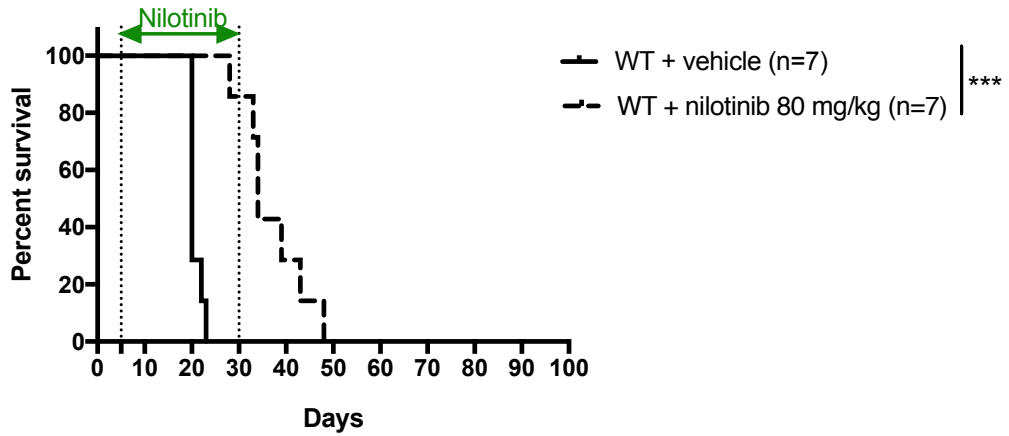
Abrogated by CD8 deficiency or IL12/TNFa blockade

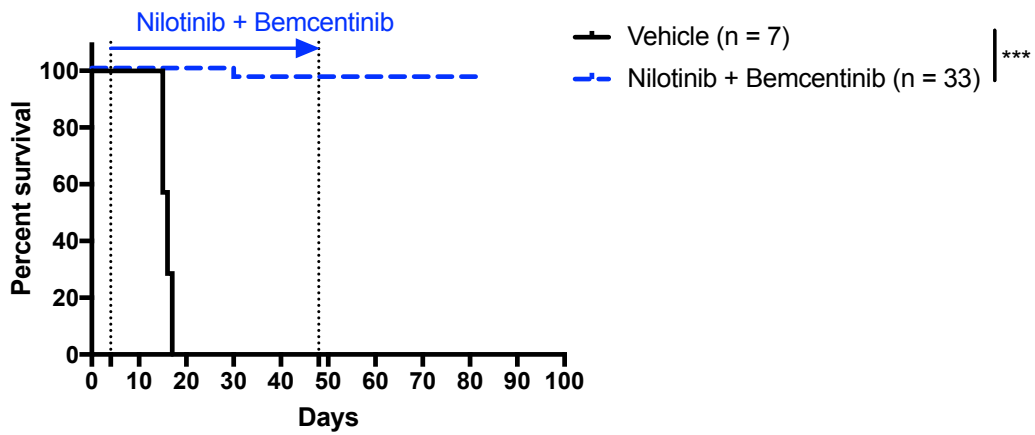
Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

..and Synergizes with Standard of Care Therapy to Eradicate Leukemia

B-ALL







Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

..in an Immune Dependent Fashion

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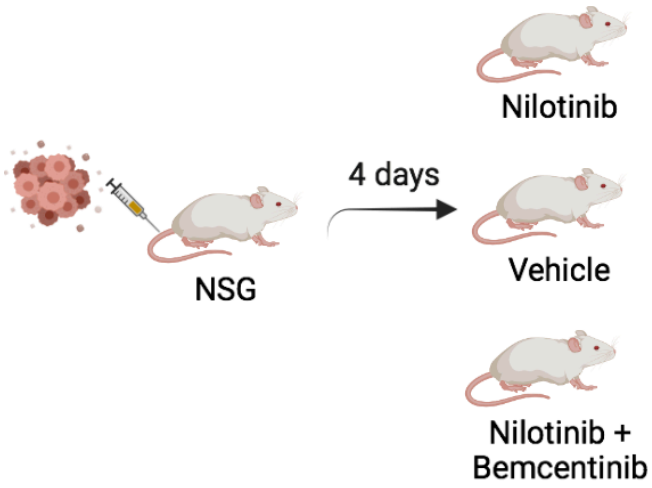

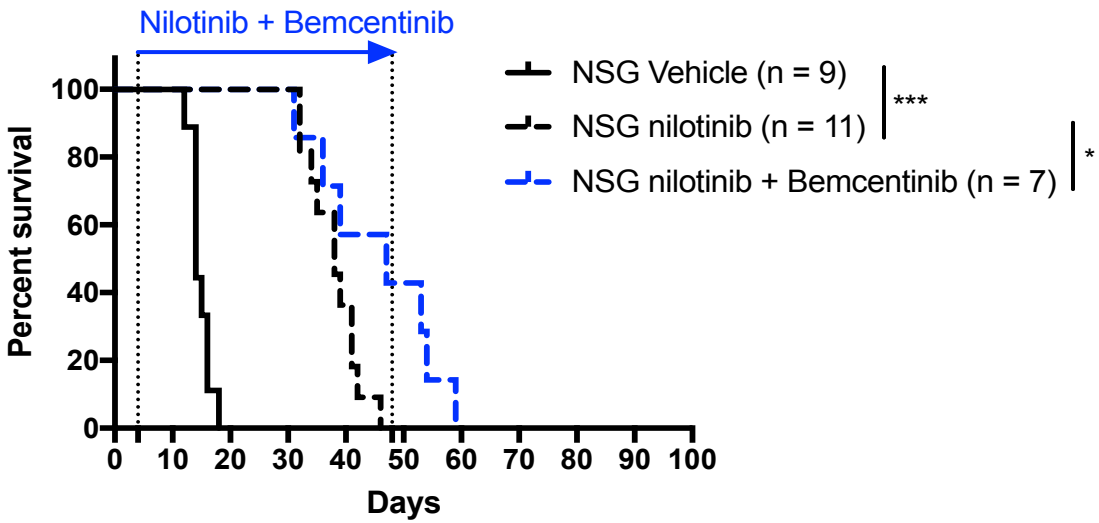

Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

AXL Inhibitor Elicits Adaptive Checkpoint Therapy upon Relapse

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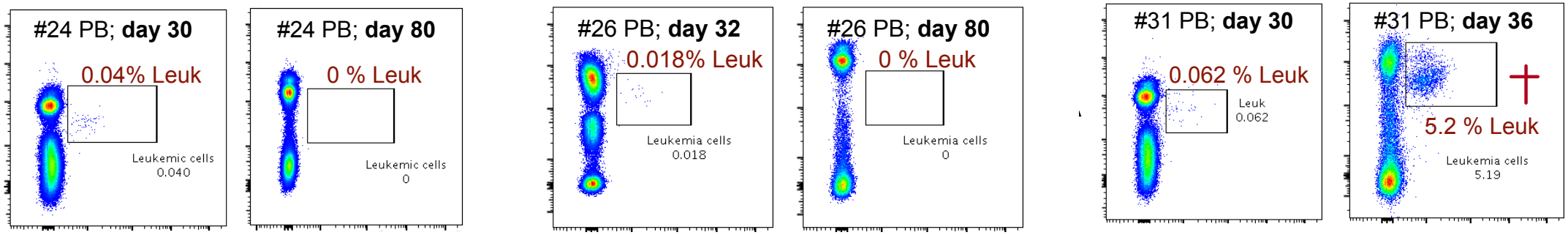
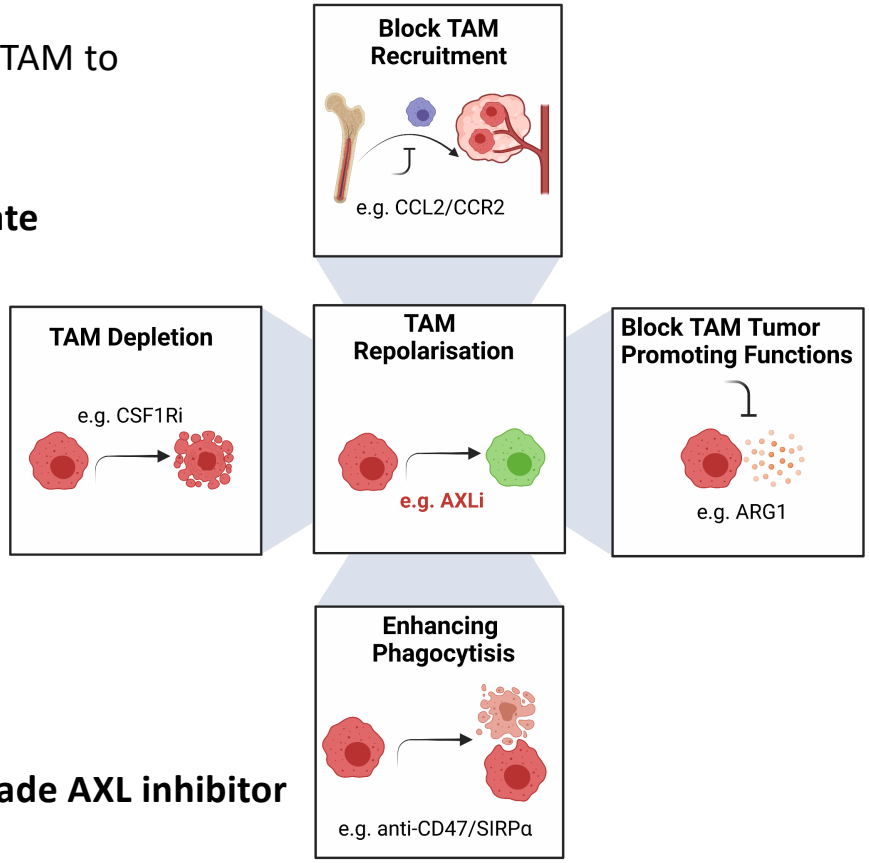


Tirado-Gonzalez, Descot, Soetopo et al, *Cancer Discovery*, 2021

Summary

- **Leukemia cells actively establish a tumor suppressive TME** by instructing TAM to produce high levels of GAS6 (IL 10 dependent process)
- **GAS6/AXL axis instructs macrophages to adopt an immune suppressive fate**
- **GAS6/AXL ablation triggers potent anti-leukemic immunity**, that depends on NK, T and cDC1.
- **GAS6/AXL ablation synergizes with standard of care therapy** to eradicate Leukemia and **promotes anti-leukemic immune memory**
- **AXL blockade elicits susceptibility to adaptive checkpoint blockade**
- These **therapeutic effects are consistently recapitulated using a clinical grade AXL inhibitor**



Acknowledgment

Medyouf Group

Irene Tirado-Gonzalez
Arnaud Descot
Alexander Schäffer
Ioanna Tsoukala
Cristóbal Santiago-Fernández
Maresa Weitmann

Alumni
Devona Soetopo
Aleksandra Nevmerzhitskaya
Sophia Thevissen
Enrico Bergonzani
Thomas Böse
Abdelrahman Mahmoud
Ewelina Czlonka
Ivan Kur

Collaborators

O. Abdel-Wahab (MSKCC, NY, USA)
C. Rothlin & S.Ghosh (Yale University, CT, USA)
J.Ghysdael & C. Tran Quang (Institut Curie, Paris, France)
K.Götze & J Slotta-Huspenina (TU Munich, Germany)
U. Platzbecker & AS Kubash (Uniklinik Leipzig, Germany)
M. Schmitz & L. Müller/ J. Meinel (TU Dresden, Germnay)
T Schmid & A. Weigert (Goethe University, Frankfurt, Germany)
H. Bonig (Goethe University, Frankfurt, Germany)
V. Benes & B. Baying &J.M. Landry (EMBL, Heidelberg, Germany)

josé CARRERAS LEUKÄMIE-STIFTUNG
erc
EMBO Young Investigator
Deutsche Krebshilfe HELFEN. FORSCHEN. INFORMIEREN.
DFG Deutsche Forschungsgemeinschaft
DKTK German Cancer Consortium