ESMO TAT

Targeted Anticancer Therapies

Bispecific γδ-T cell engagers for the treatment of cancer

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ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

DECLARATION OF INTERESTS

Grant/Research support from: Glycostem, LAVA Therapeutics

Stockholder in: LAVA Therapeutics

Employee of: LAVA Therapeutics and Amsterdam UMC

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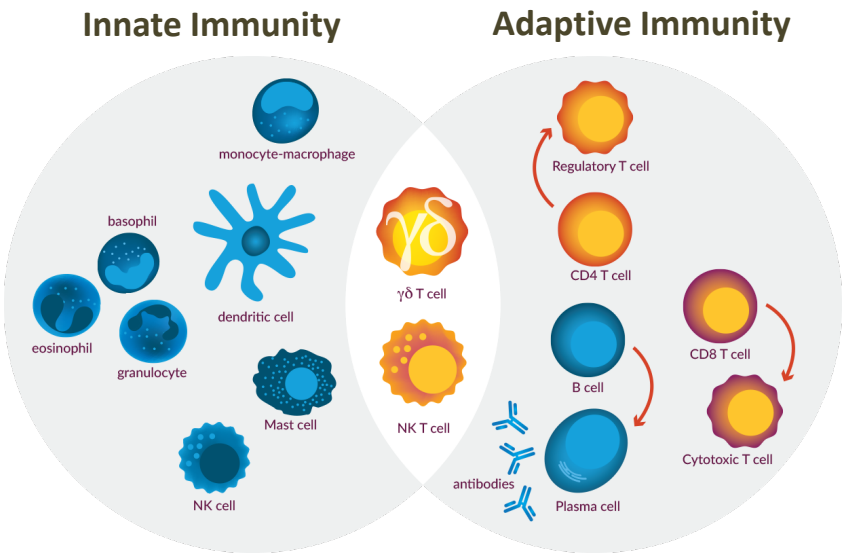
γδ-T cells play a central role in antitumor immunity



Adapted from Dranoff G., Nat Rev. Cancer 2004; 4: 11-22

Vδ2-T cells

- **largest γδ-T cell subset:** ~90-95% in peripheral blood
- monomorphic TCR: **Vδ2 preferentially pairs with Vγ9**
- **well defined specificity:** phosphoantigen-BTN2A1/3A1 complex
- consistent **proinflammatory cytotoxic** effector T cell population
- natural ability to recognize and kill tumor cells
- unique **antigen presenting ability**
- **positive association** with outcome **in cancer** patients

Vδ1-T cells

- infrequent in blood, more prevalent in **mucosa/epithelia**
- diverse TCR repertoire: **Vδ1 pairs with multiple Vγ chains and αβ-TCR**
- **diverse specificity:** different antigen presenting molecules and antigens
- **functionally diverse:** cytotoxic, protumor, and regulatory (IL-10, IL-17)
- **variable association** with outcome **in cancer** patients

Yeung MM, et al. Gut 2000; 47:215
Kimura Y, et al. Cancer Sci 2016; 107:1206
Lo Presti, et al, Front Immunol 2014; 5:1
Pang DJ, et al. Immunology 2012; 136:283
Adams EJ, et al. Cell Immunol 2015; 296:31
Siegers GM, et al. Mol Ther 2014; 22:1416
Wu P, et al. Immunity 2014;40:785
Adams EJ, et al. Annu Rev Immunol 2013; 31:529
Lo Presti E, et al. Cancer Immunol Res 2017; 5:397

γδ T cells belong to the first line of defense against cancer

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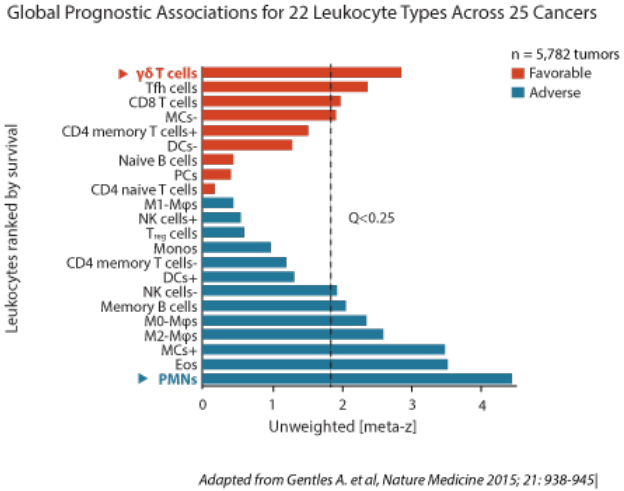
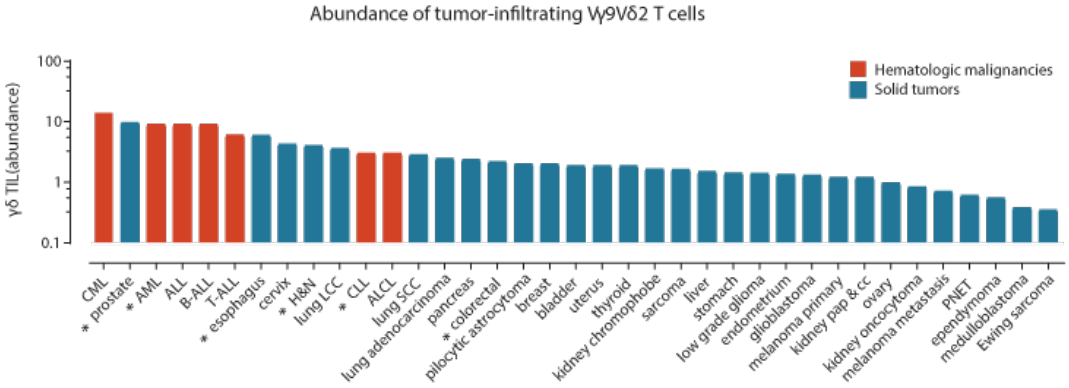
Presence of Vγ9Vδ2-T cells in tumor tissue shown to correlate with favorable prognosis for cancer patients



Adapted from Gentles A. et al, Nature Medicine 2015; 21: 938-945|



**: in vitro/ex vivo* data generated using LAVA's γδ-bsTCEs

Adapted from Tosolini M et al. Oncoimmunology 2017, vol 6, e128472

γδ-T cells most strongly correlated with favorable outcome of leukocyte subsets analyzed

Vγ9Vδ2-T cells exist as tumor-infiltrating lymphocytes (TILs) in both hematologic malignancies and solid tumors

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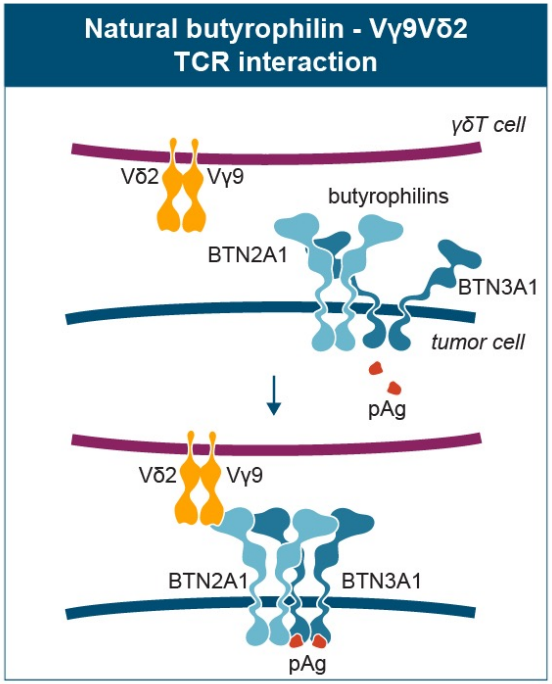
Vγ9Vδ2-T cells naturally respond to pAg-butyrophilin complexes and have strong antitumor activity

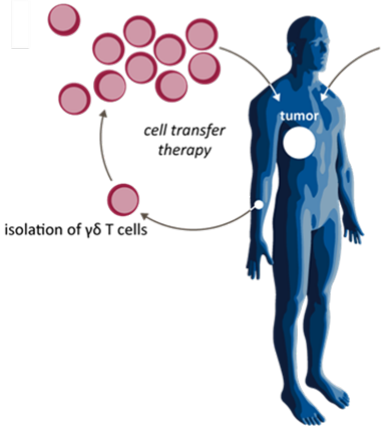


- **Predominant γδ-T cell subset in peripheral blood**
  - 1-10% of all T-cells in circulation
- **Vγ9Vδ2-TCR is monomorphic and specific for phosphoantigen-bound butyrophilin (BTN)3A1 / BTN2A1**
  - pAg are intermediates of mevalonate metabolic pathway (e.g. IPP) and of microbial DOXP pathway (e.g. HMBPP)
  - Vγ9Vδ2-T cells function in an MHC-unrestricted manner
- **Pro-inflammatory, effector & APC functions**
- **Important role in immune surveillance**
- **Cytotoxic against tumor cells with increased pAg levels due to metabolic dysregulation**

Vyborova A, et al. J Clin Invest 2020; 130: 4637-4651
Rigau M, et al. Science 2020; 367: 6478
Karunakaran MM, et al. Immunity 2020; 52: 487-498

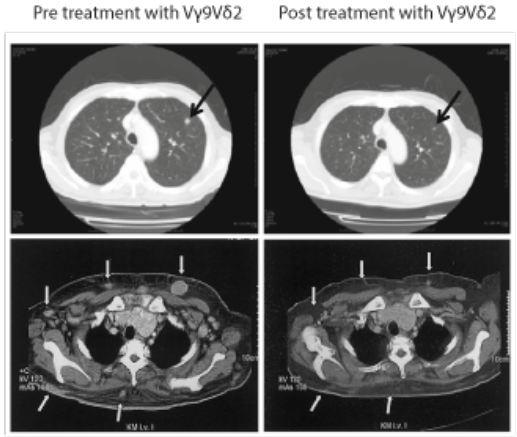
Clinical Vγ9Vδ2-T cell based therapeutic approaches demonstrate therapeutic potential and safety

***ex vivo* activation**



***in vivo* activation**

Systemic activation and proliferation via treatment with Vγ9Vδ2 T cell ligand (BrHPP or NBP ± IL-2)



Kobayashi H, et al. Cancer Immunol Immunother 2011; 60:1075-1084
Wilhelm M, et al. Blood 2003;102:200-206

- Clinical trials performed with *in/ex vivo* activation protocols showed promising objective responses and safety
- No signs of cytokine release syndrome (CRS) as a result of Vγ9Vδ2-T cell activation

Inherent potential and safety of Vγ9Vδ2 T cell-based therapy in cancer demonstrated
Can stronger and more consistent antitumor responses be achieved using a bispecific Ab based approach?

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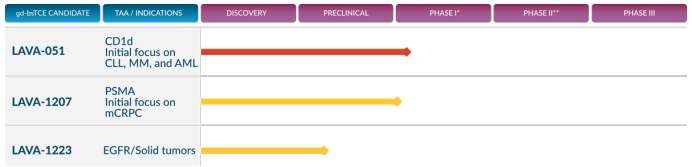
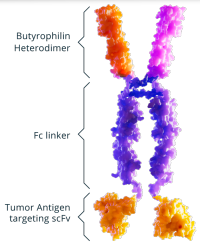
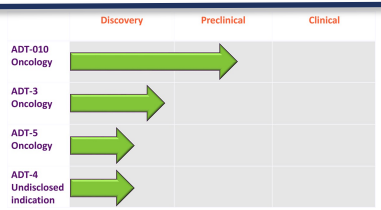
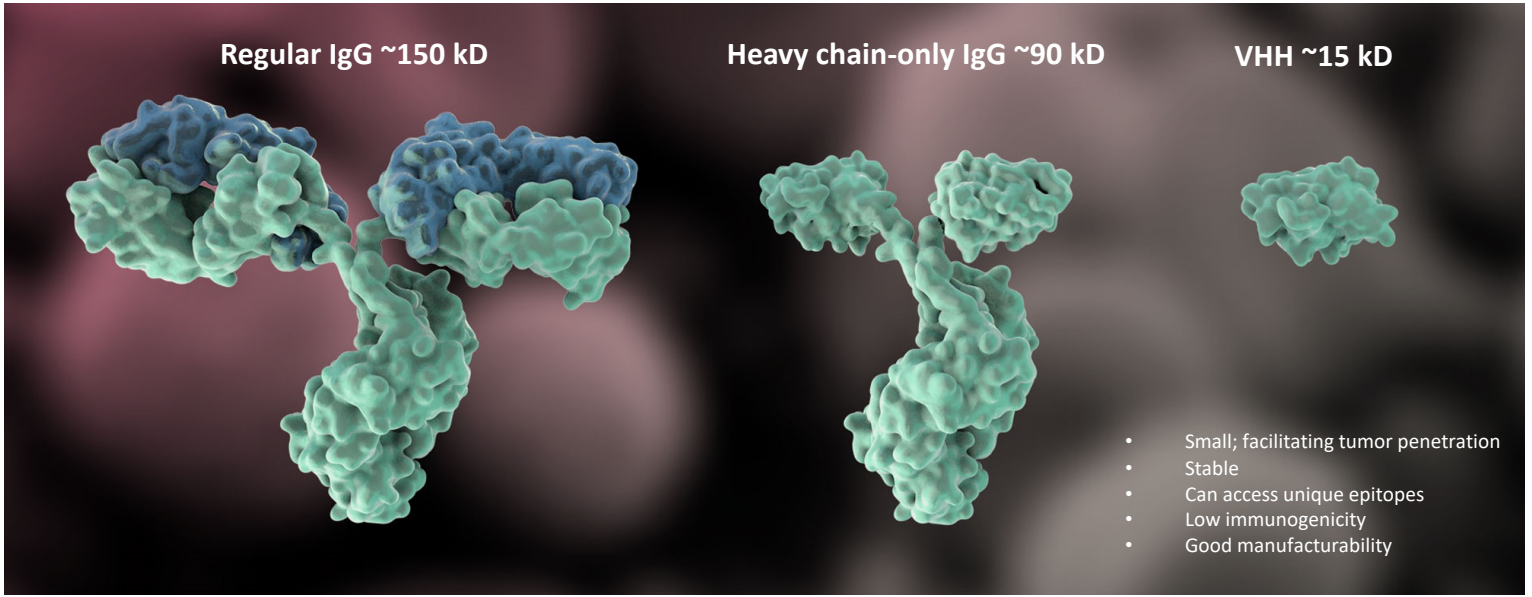
Companies developing bispecific antibodies to target γδ-T cells

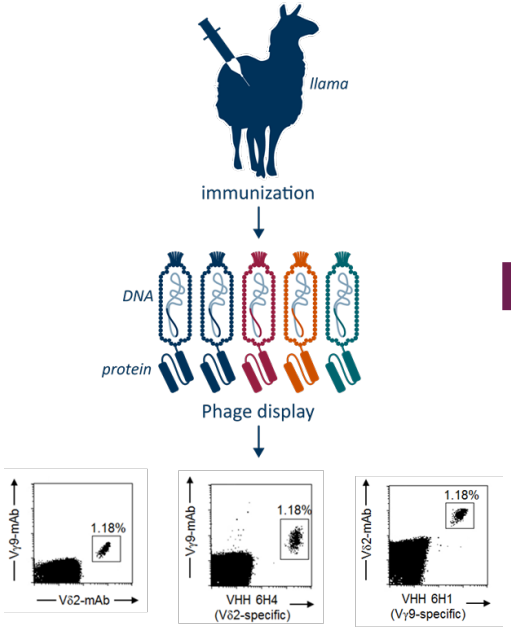
company	γδ-subset	format(s)	pipeline	stage
LAVA THERAPEUTICS	Vδ2			clinical
SHATTUCK LABS	Vδ2			preclinical
aδaptate Takeda	Vδ1	not disclosed		preclinical

Single domain antibodies/VHHs



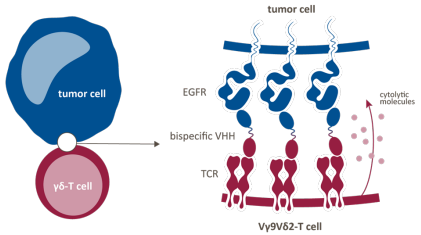
Adapted from Leslie M. Science 2018; 360:594-597

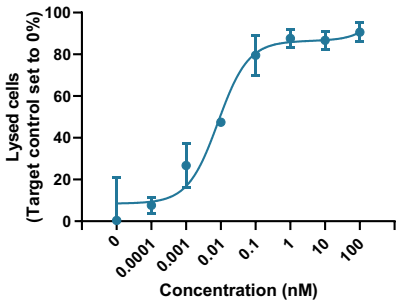
Generation of Vγ9Vδ2-TCR specific VHHs and bispecific VHHs

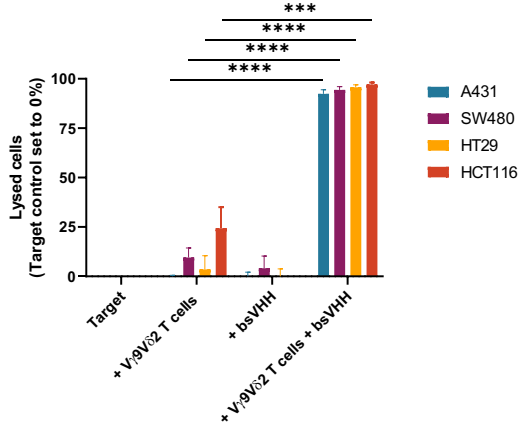


selection of VHH specific for Vγ9 or Vδ2-TCR

EGFR-γδ bsVHHs trigger potent Vγ9Vδ2-T cell lysis of EGFR+ tumor cells
- regardless of RAS/BRAF mutations -







de Bruin RC, et al. Clin Immunol 2016; 169:128-38
de Bruin RC, et al. Oncoimmunology 2017; 7:e1375641
King L, et al. manuscript in preparation.

LAVA develops two bispecific Vγ9Vδ2-T cell engaging formats

gd-bsTCE CANDIDATE	TAA / INDICATIONS	DISCOVERY	PRECLINICAL	PHASE I*	PHASE II**	PHASE III
LAVA-051	CD1d Initial focus on CLL, MM, and AML			trial recruiting		
LAVA-1207	PSMA Initial focus on mCRPC			trial recruiting		

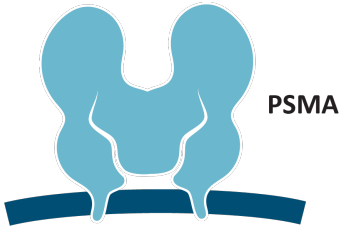
Bispecific single domain antibody

- High affinity binding and high potency
- Smaller molecule than regular IgG1 (~ 30kD)
- Short *in vivo* half-life, prolonged functional half-life
- Used for lead hematological program (LAVA-051)

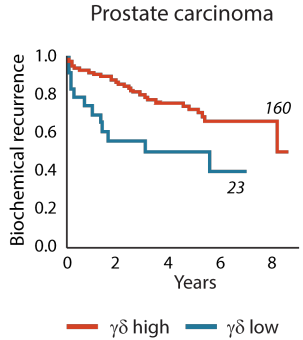
Bispecific single domain antibody with Fc domain

- High affinity binding and high potency
- Smaller molecule than regular IgG1 (~ 80kD)
- *In vivo* half-life similar to regular IgG1
- Validated mutations to silence Fc effector function
- Used for lead solid tumor program (LAVA-1207)

Prostate Specific Membrane Antigen (PSMA)

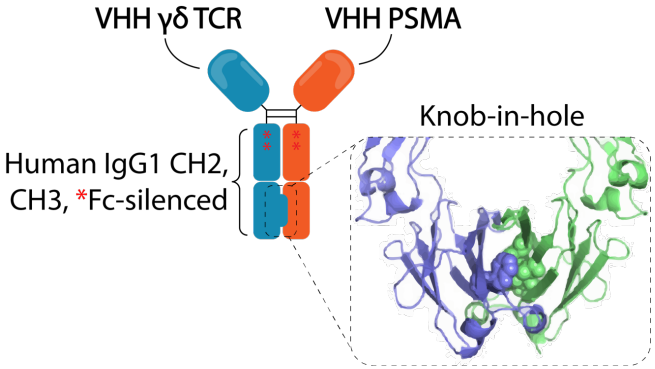


- **Significantly overexpressed in nearly all primary and metastatic prostate cancer tissues**
  - Low expression in normal human tissue (prostate, small intestine, proximal renal tubules, and salivary glands)
- **Prostate cancer remains a major area of unmet medical need**
  - 5-year survival rate for mPC is 30%; an estimated >34K men died of mCRPC in the US in 2020
- **Prostate cancer has relatively high abundance of tumor-infiltrating Vγ9Vδ2-T cells**
- **Tumor-infiltrating Vγ9Vδ2-T cell abundance correlates with improved patient outcome**

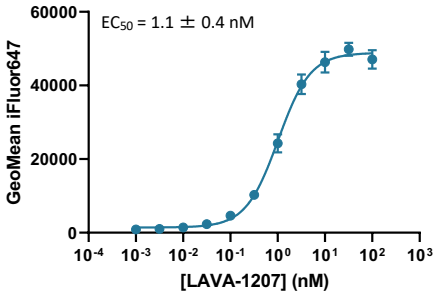


Bostwick et al. 1998, Cancer 82:2256-61
Sweat et al. 1998, Urology 52:637-640
Abstract 1773 AACR Annual Meeting 2018
https://seer.cancer.gov/statfacts/html/prost.html
Tosolini et al. 2017, Oncoimmunology 6:e1284723

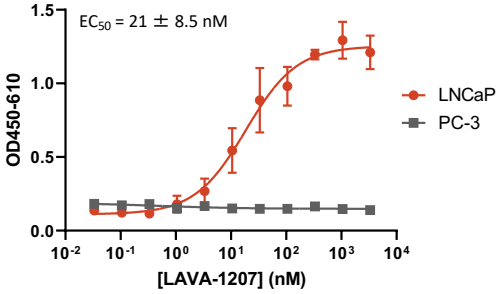
PSMA-Vδ2 bispecific VHH-Fc (LAVA-1207)



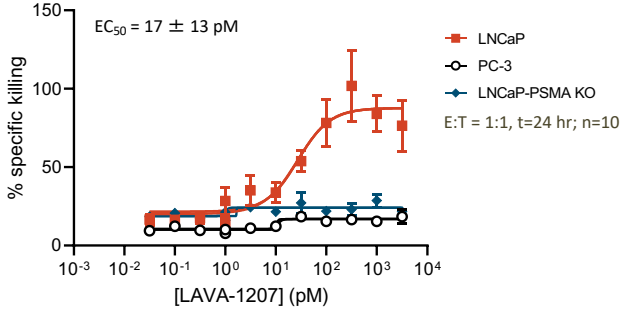
Vγ9Vδ2-T cell binding



PSMA binding



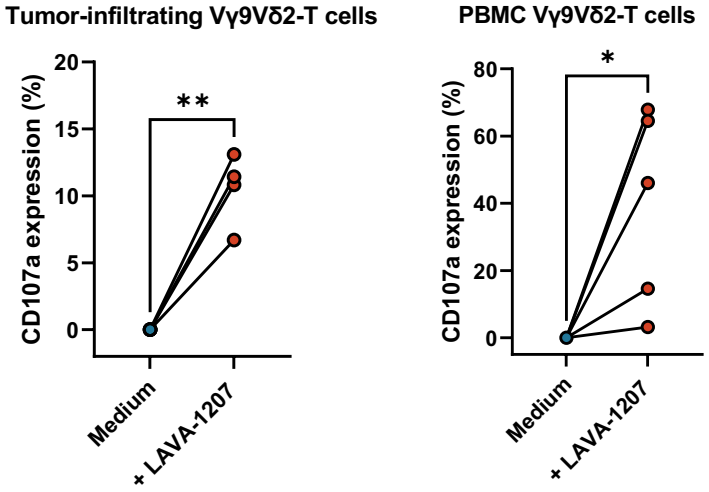
Cytotoxicity



King L, et al. manuscript in preparation

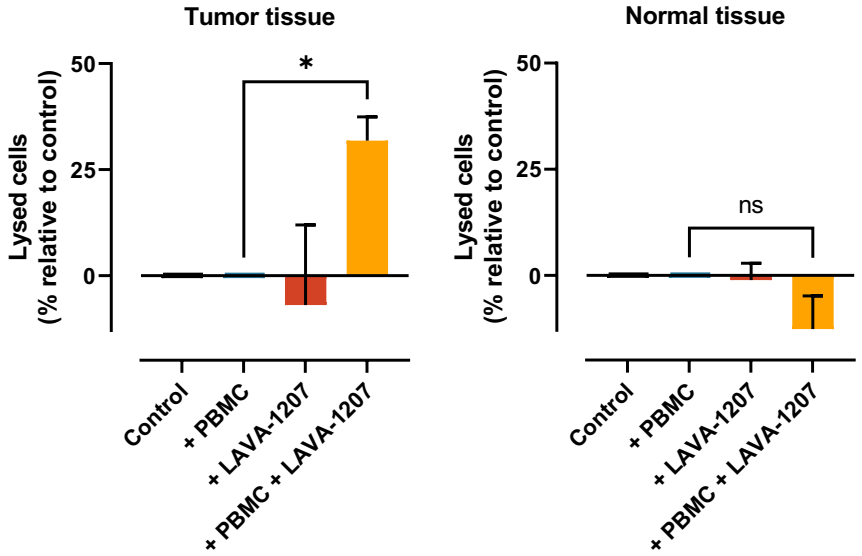
LAVA-1207 triggers prostate cancer patient Vγ9Vδ2-T cells to degranulate and lyse autologous tumor cells

Vγ9Vδ2-T cell degranulation



Prostate cancer pt derived tumor suspensions were cultured 4 hr in the absence (left) or presence (right) of autologous PBMC ± 50nM LAVA-1207 (*P <0.05; **P <0.01)

Preferential lysis of prostate tumor cells

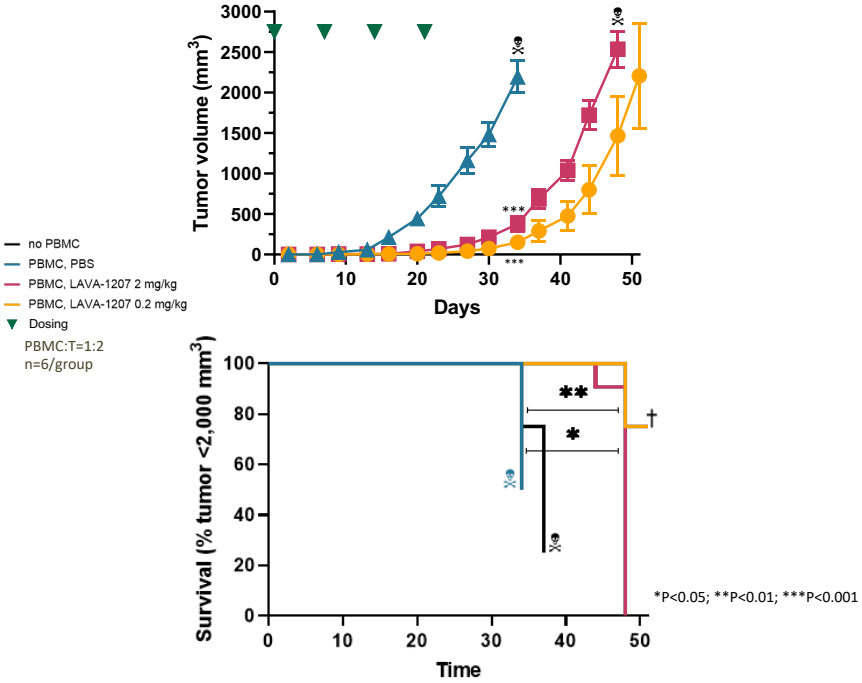


Prostate cancer pt derived tumor and normal prostate tissue cultured 24 hr with autologous PBMC (PBMC:T=10:1) ± 50nM LAVA-1207 (mean ± SEM; *P <0.05; n=3)

King L, et al. manuscript in preparation

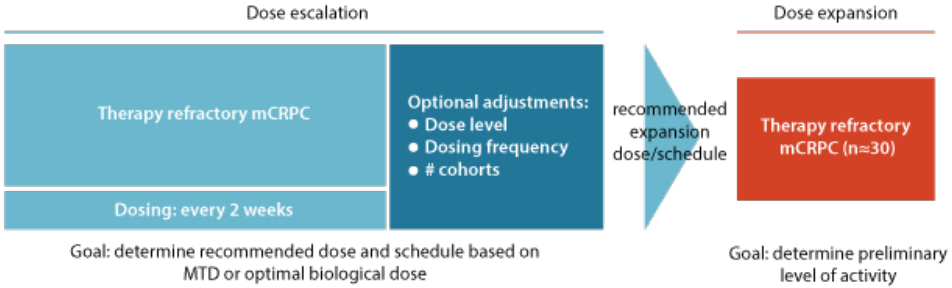
LAVA-1207: *in vivo* antitumor activity and phase 1/2a trial design

Subcutaneous 22Rv1-human PBMC admixed *in vivo* model



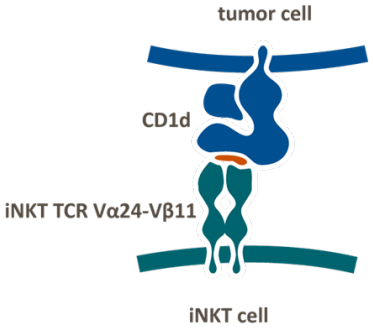
King L, et al. In preparation

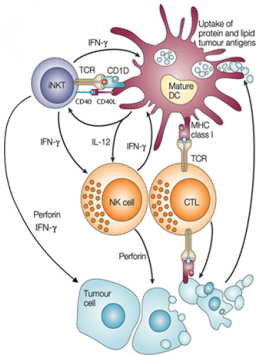
Trial design



Cluster of differention 1d (CD1d)

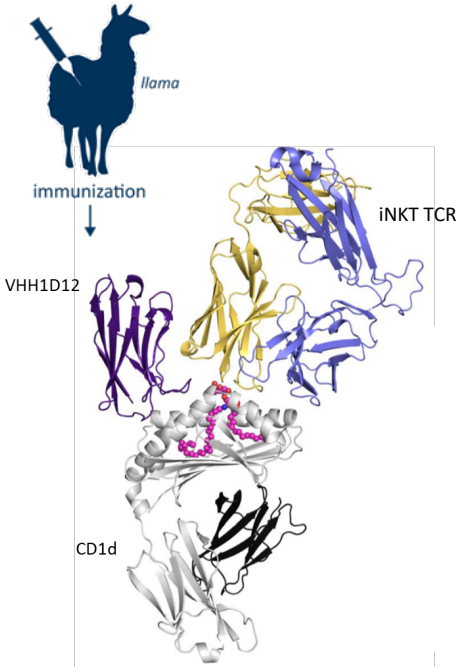
- **CD1d: MHC class I-related glycoprotein that presents glycolipid antigens to iNKT cells**
- **CD1d is expressed on the surface of various human antigen presenting cells including DCs and B-cells**
- **Indications of interest include:**
  - Hematological indications: e.g. CLL, AML, T-ALL and MM
  - Solid tumor indications: CRC, lung, head & neck, breast, renal, melanoma and neuroblastoma
- **Expressed by immunosuppressive cells in the tumor (MDSC and TAM)**
- **CD1d-iNKT axis-directed therapies demonstrated a favorable toxicity profile**





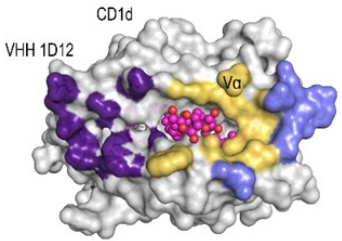
Canchis PW, et al, Immunology 1993; 80:561-565; Metelitsa LS. Clin Immunol 2011; 140:119-129;
King L, et al. Front Immunol 2018; doi: 10.3389/fimmu.2018.01519; Wilson SB, et al. Nat Rev Immunol 2003;3:211-222

CD1d-specific VHH1D12 triggers iNKT cell activation through intrinsic bispecificity



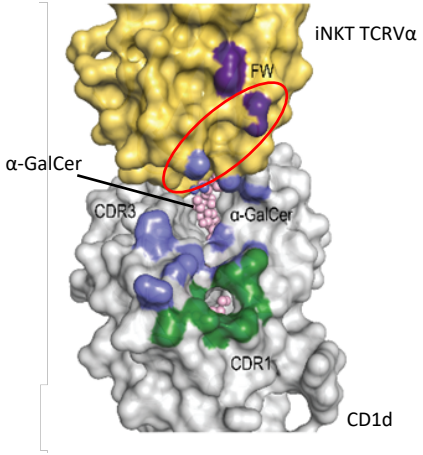
Top view of CD1d

footprints of CD1d-VHH and iTCR

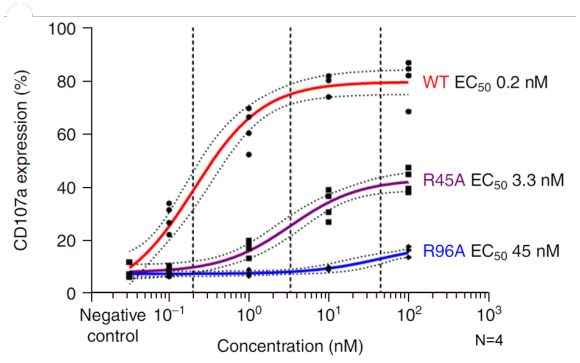


Side view of CD1d-iNKT TCRVα

footprint of CD1d-VHH



iNKT degranulation critically depends on CD1d-VHH R96 and R45



Expression of CD107a on iNKT after a 4 hr co-culture of expanded iNKT with CD1d+ MM.1s cells ± 10 nM or a concentration range of CD1d-VHH1D12 and alanine mutants (n=4)

Lameris R, et al. Nature Cancer 2020;1:1054-1065

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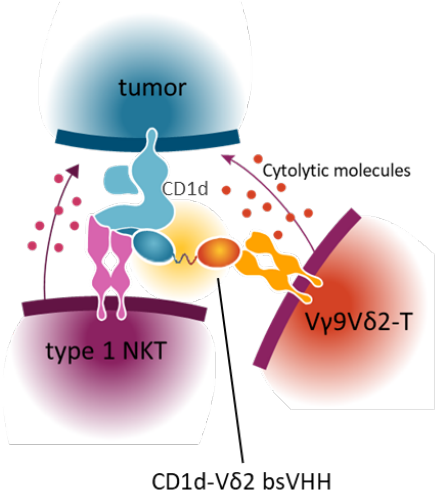
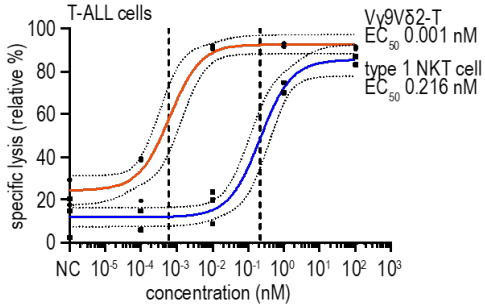
CD1d-Vδ2 bispecific VHH (LAVA-051) stimulates both iNKT and Vγ9Vδ2-T cell effector functions and proliferation

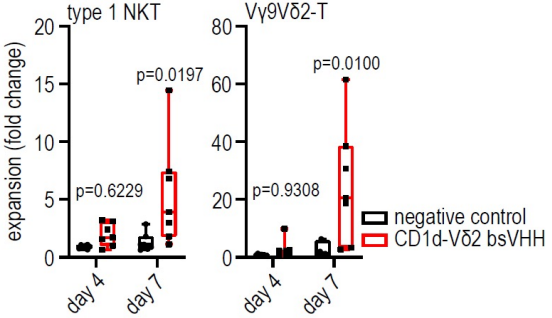


cytotoxicity



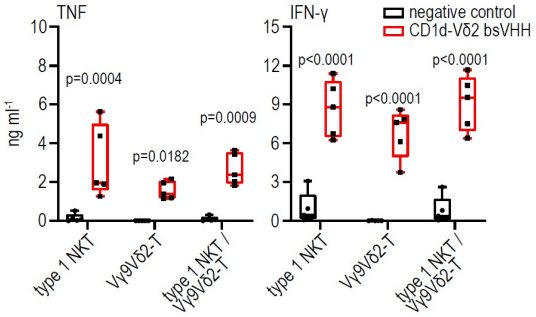
Lysis of CCRF-CEM by type 1 NKT and Vγ9Vδ2-T ± CD1d-Vδ2 bsVHH (t=16hr)

expansion



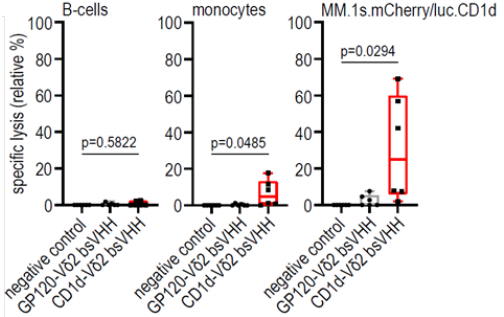
Expansion of iNKT and Vγ9Vδ2-T cells in 7 day culture of PBMC and CD1d+ MM.1s cells ± 50nM CD1d-Vδ2 bsVHH (PBMC:target ratio of 10:1).

cytokine production



Cytokine secretion by iNKT, Vγ9Vδ2-T or a mix (ratio 1:1, 5x10⁴ total effector cells) after 24 h culture with CD1d+ MM.1s cells ± 50nM CD1d-Vδ2 bsVHH

preferential lysis of CD1d+ tumor



Lysis after overnight culture of PBMC and CD1d+ MM.1s cells (ratio 10:1) ± 50nM gp120-Vδ2 bsVHH or CD1d-Vδ2 bsVHH. Specific lysis relative to negative control (n=6)

Lameris R, et al. manuscript in preparation

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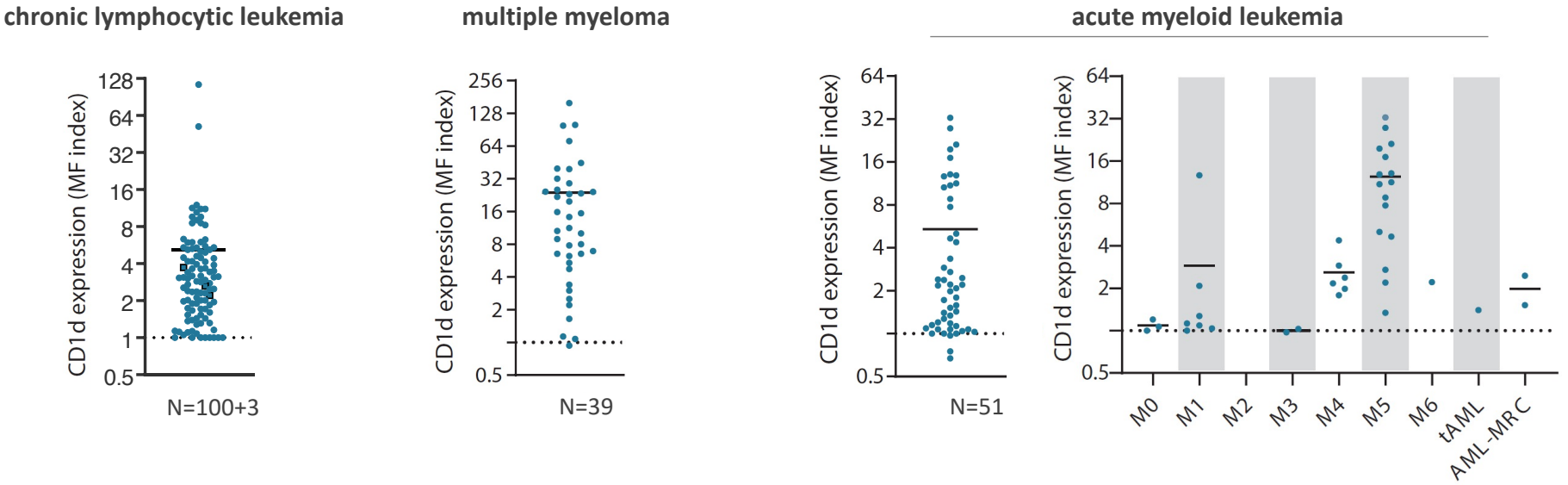
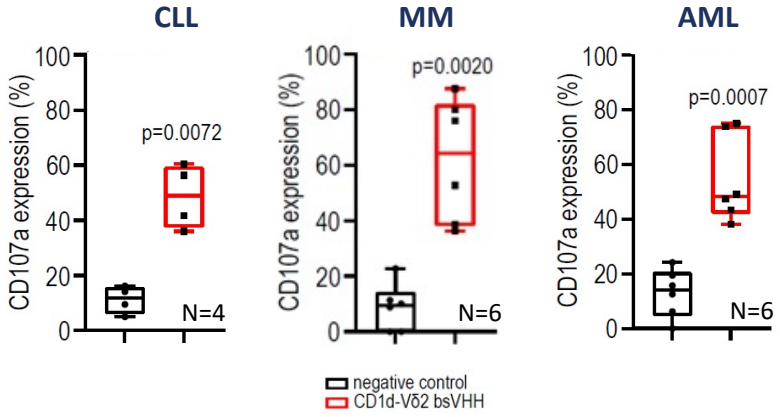
CD1d is expressed on tumor cells of patients with CLL, MM and AML



Lameris R, et al. manuscript in preparation

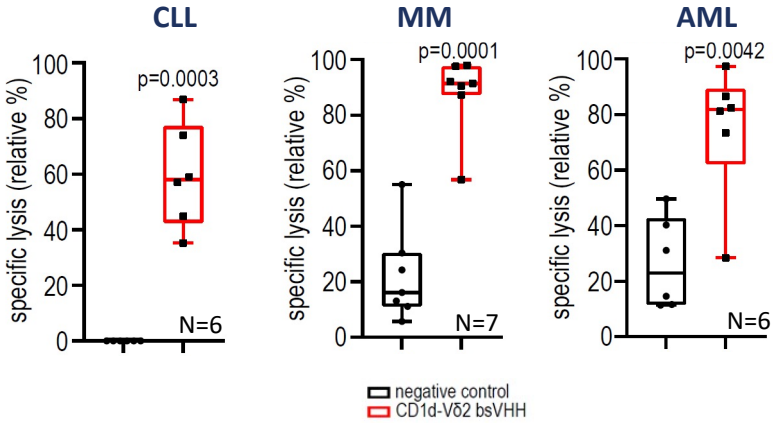
Activity of LAVA-051 in patient tumor samples of CLL, MM and AML

Degranulation of patient Vγ9Vδ2-T cells



Expression (%) of CD107a on autologous Vγ9Vδ2-T cells after 16hr coculture of patient samples (PBMC (CLL) or bone marrow (MM and AML)) ± CD1d-Vδ2 bsVHH (50 nM), analysed by flow cytometry.

Lysis of patient tumor cells

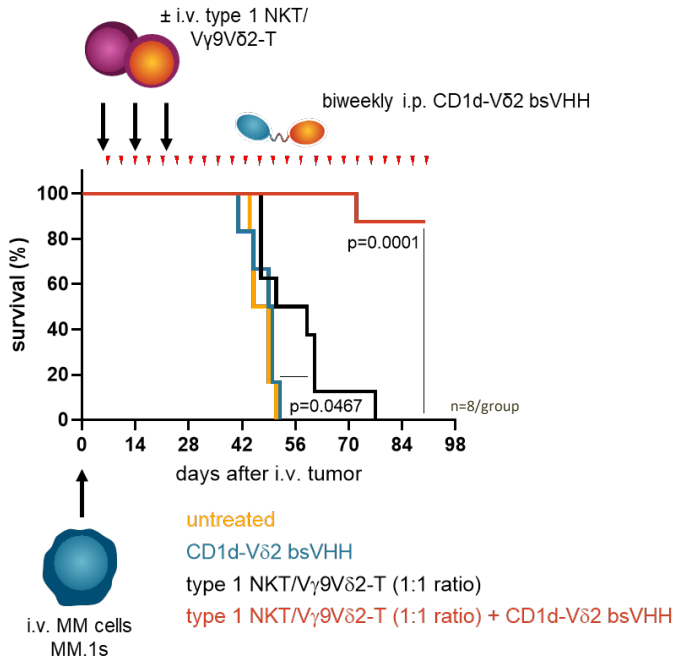


Cytotoxicity towards patient CLL, MM, and AML cells after 16 hr coculture of iNKT and Vγ9Vδ2-T cells (1:1 mix) and patient PBMC or BMMC (E:T=1:2) plus negative control (NC) or 50nM CD1d-Vδ2 bsVHH; quantified by flow counting beads; expressed relative to tumor cells only.

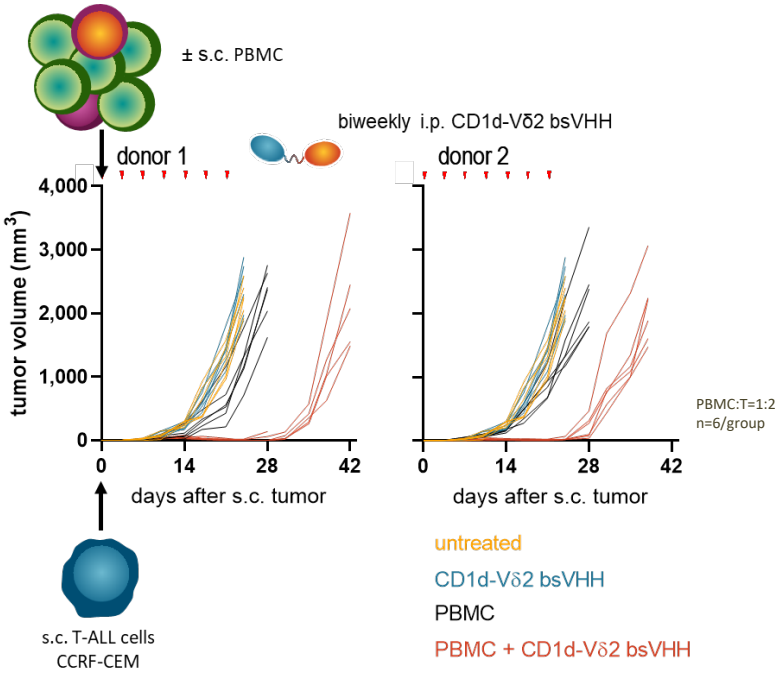
Lameris R, et al. manuscript in preparation

LAVA-051 induces antitumor activity *in vivo* and increases survival

Intravenous Multiple Myeloma model



Subcutaneous T-ALL model



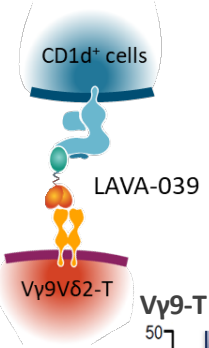
Hans van der Vliet MD, PhD

Lameris R, et al. manuscript in preparation

Exploring the safety of γδ-T cell engagement in non-human primates

-LAVA-051: -CD1d specific VHH1D12; **not** NHP cross-reactive
-Vδ2-TCR specific VHH; **not** NHP cross-reactive

-LAVA-039: -CD1d specific VHH1D22; NHP cross-reactive
-Vγ9-TCR specific scFv; NHP cross-reactive

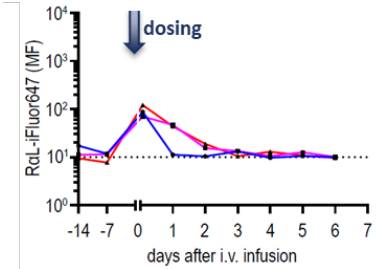


→ ***LAVA-039 explored in Macaca fascicularis***

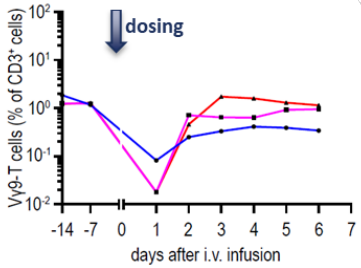
Single dose: 0.1 mg/kg; 0.3 mg/kg; 1 mg/kg (i.v.; n=1/dose)

Multiple (7) daily doses: 0.1 mg/kg; 0.3 mg/kg; 1 mg/kg (i.v.; n=1/dose)

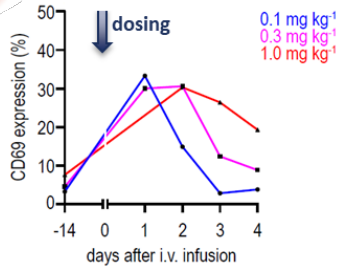
compound binding to Vγ9-T cells



Vγ9-T cell frequency

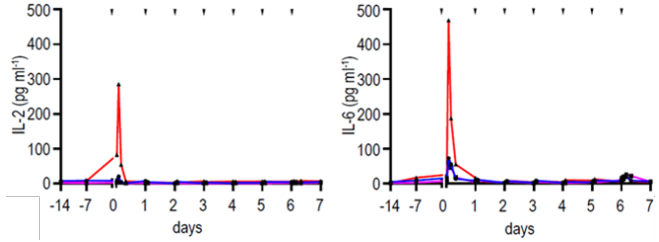


Vγ9-T cell activation

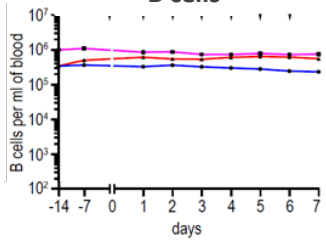


-no clinical signs of toxicity
-no clinical chemistry abnormalities
-no histopathological abnormalities
-no depletion of CD1d+ B cells/monocytes
-low cytokine spike
-bispecific γδ-TCE detectable on PB and LN γδ-T

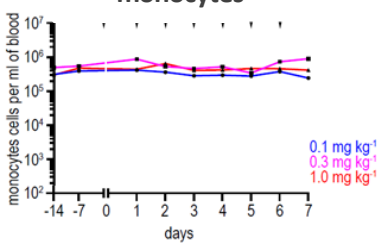
cytokine levels



B cells



monocytes



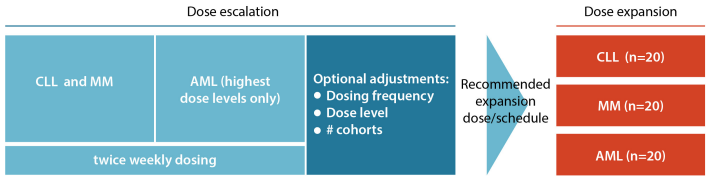
ESMO TAT

Hans van der Vliet MD, PhD

Lameris R, et al. manuscript in preparation

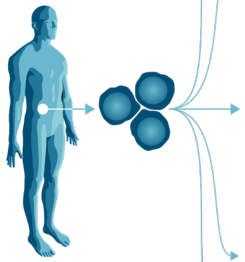
LAVA-051 first-in-human phase 1/2a study

Trial design



ClinicalTrials.gov Identifier: NCT04887259

Extensive monitoring

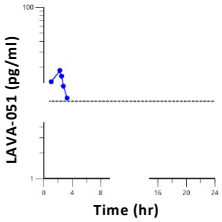
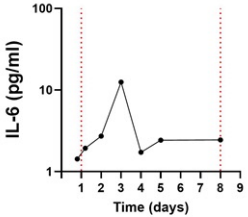
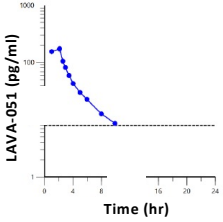
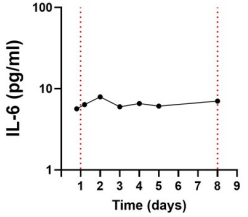
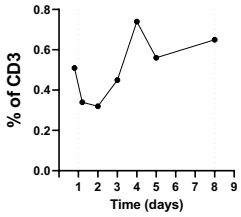
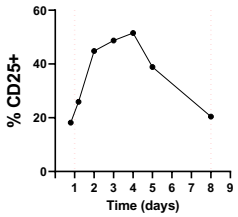
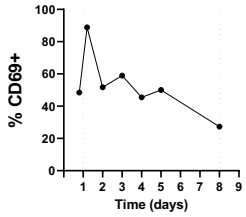
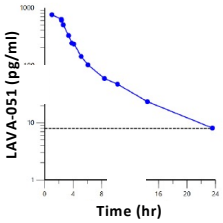
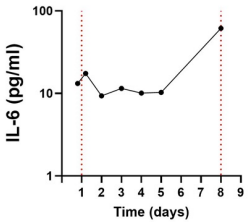
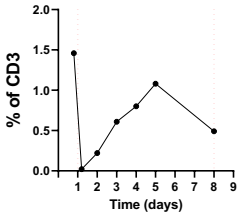
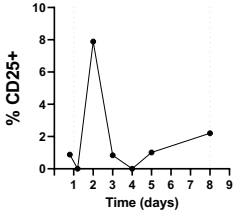
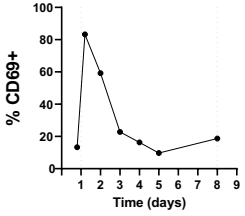


Pharmacokinetics			
Anti-Drug Antibodies			
Pharmacodynamics	1. Cytokines (IL-1β, IL-2, IL-6, IL-8, TNF-α, IFN-γ, GM-CSF)		
	2. Flow cytometry	a.	Binding of LAVA-051: Vγ9Vδ2-T cells | CD1d positive tumor cells
		b.	Activation status & frequency: Vγ9Vδ2-T cells | iNKT cells
		c.	Induction of activation of Vγ9Vδ2-T cells *ex vivo* when exposed to CD1d (functional assay)
		d.	Immune monitoring (frequency and activation status of B cells, T cell subsets, NK cells, monocytes, dendritic cells)
Disease assessments	Tumor defining markers/CD1d/BTN3A		- MM (peripheral blood, urine, CT scan, bone marrow biopsy) - CLL (peripheral blood, CT scan, bone marrow biopsy) - AML (peripheral blood, bone marrow biopsy)
Safety	Chemistry / hematology / urine		

→ **Initial observations of first 3 single patient cohorts**

Hans van der Vliet MD, PhD

LAVA-051 phase 1 study: first observations

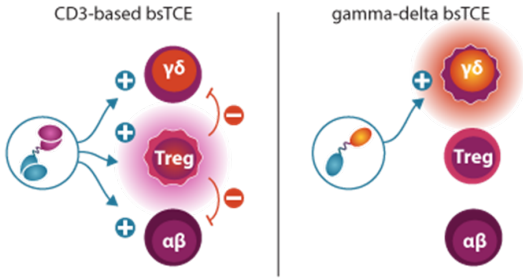
Cohort	Dose	PK	Cytokines (IL-6)	LAVA-051 max RO	Vγ9Vδ2 T cells: frequency	Vγ9Vδ2 T cells: activation markers (CD25, CD69)		Clinical
1	0.45 µg			n.d.	n.d.	n.d.	n.d.	**Multiple Myeloma** No CRS/DLT
2	3 µg			2.8%				**CLL** No CRS/DLT
3	15 µg			5.6%				**CLL** No CRS/DLT On study; *SD* at 12wk *Multiple enlarged painful (diseased) lymph nodes (@ 1 wk, subsequent regression); reminiscent of tumor flare*

clinical data cut-off date for presented patients: Dec 15, 2021
day 1 = start of treatment

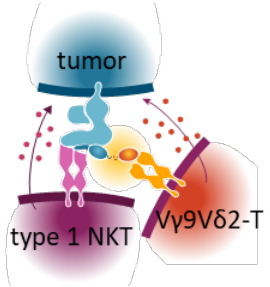
= dosing

Conclusions

- **Strong scientific and clinical rationale for tumor targeted engagement of Vγ9Vδ2-T cells**
- **Bispecific γδ-T cell engagers differentiate from CD3 (pan) T cell engagers**
  - Vγ9Vδ2-T cell restricted activation expected to result in a more benign toxicity profile
  - Vγ9Vδ2-T cell engagers avoid co-activation of immunosuppressive Tregs
- **Bispecific Vγ9Vδ2-T cell engagers are studied in 2 ongoing clinical phase 1/2a studies**
  - Several other γδ-T cell engagers in preclinical development



- **LAVA-1207: humanized PSMA-Gammabody™**
  - first-in-human clinical phase 1/2a study initiated in patients with therapy refractory mCRPC
- **LAVA-051: humanized CD1d-Gammabody™**
  - triggers CD1d-restricted lysis via *dual* activation of iNKT cells and Vγ9Vδ2-T cells
  - first-in-human clinical phase 1/2a study initiated in patients with CLL, MM, or AML that are refractory to prior therapy
  - Data from initial 3 dose cohorts showed that LAVA-051 was well tolerated early in dose escalation with on-mechanism pharmacodynamics consistent with Vγ9Vδ2-T cell engagement



Acknowledgements

LAVA THERAPEUTICS

Jurjen Ruben
Sigrid Ruuls
Rob Roovers
Thilo Riedl
Victoria Iglesias
Lisette Bevaart
Peter Machielsen
David Lutje Hulsik
Pauline van Helden
Jessica Truscello
Sanjana Umarale
Ilse Tuinhof
Jorden Veeneman
Steve Hurly
Ton Adang
Benjamin Winograd
Paul Parren

Amsterdam UMC
Universitair Medische Centra

Roeland Lameris
Lisa King
Milon de Jong
Jose Saura Esteller
Myrthe Veth
Jana Vree
Iris de Weerdt
Sonja Zweegman
Niels van de Donk
Arnon Kater
Jens Voortman
Tanja de Gruijl

Erasmus MC
University Medical Center Rotterdam

Annemiek Broijl
Martijn Lolkema

THE UNIVERSITY OF MELBOURNE

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Jamie Rossjohn lab