(Overcoming) New challenges in the blood bank: anti-CD47, a new agent for targeted cancer therapy and the next generation of anti-CD38 therapeutics

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Objectives

1. CD47
2. Update on CD38
3. Comparison of anti-CD47 and anti-CD38 in the blood bank

Current CD47-Targeting Agents

Agent	Developer	Description	Tumor Types	Binds to RBCs
Hu5F9-G4	Forty Seven Inc.	Humanized monoclonal anti-CD47	Solid, AML,MDS	Yes
CC-90002	Celgene	Humanized monoclonal anti-CD47	AML, MDS	Yes
TTI-621	Trillium Therapeutics	Anti-SIRPα recombinant fusion protein	Numerous solid and hematologic	No
ALX148	Alexo Therapeutics	CD47-SIRPα blocking fusion protein	Solid, lymphoma	No data reported
SRF231 *New*	Surface Oncology	Human monoclonal anti-CD47	Solid, CLL, lymphoma	Pre-clinical

- **mAb anti-CD47**
  - Hu5F9-G4 – IgG4 to CD47
  - CC-90002 – IgG4 to CD47
- **Fusion Proteins**
  - TTI-621
    - IgG1 immune checkpoint inhibitor targets CD47 binding domain of SIRPα
    - Soluble decoy receptor
  - ALX148
    - Inactive Fc targeting binding domain of SIRPα
    - Blocking agent
- May be used with other immunomodulary agents (IMiDs) in combination therapies

Clinical Trial Landscape of CD47-Targeting Agents

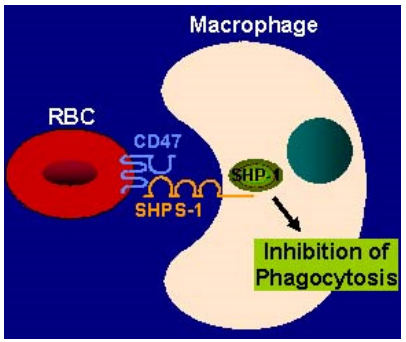
Trial Drug	Phase	Condition	Intervention	Location
Hu5F9-G4	1	AML, MDS	Hu5F9-G4 monotherapy	UK
Hu5F9-G4	1	AML, MDS	Hu5F9-G4 monotherapy or Hu5F9-G4 plus azacitidine (VIDAZA)	USA
Hu5F9-G4	1	Colorectal neoplasm	Hu5F9-G4 plus cetuximab (Erbitux)	USA
	2 ←	Solid malignancy		
Hu5F9-G4	1	Solid malignancy	Hu5F9-G4 monotherapy	USA
Hu5F9-G4	1	NHL	Hu5F9-G4 plus rituximab (Rituxan)	USA, UK
	2 ←	Indolent or B-cell Lymphoma		
CC-90002	1	AML, MDS	CC-90002 monotherapy	USA
CC-90002	1	NHL	CC-90002 plus rituximab (Rituxan)	USA, Spain
TTI-621	1	Leukemia, Lymphoma, Multiple myeloma, MDS, Small cell lung cancer	CC-90002 monotherapy, CC-90002 plus rituximab (Rituxan) or CC-90002 plus nivolumab (Opdivo)	USA, Canada
TTI-621	1	Numerous solid malignancies: melanoma to breast cancer	TTI-621 monotherapy (relevent PD-1/PD-L1 inhibitor may be used in combination)	USA
ALX148	1	NHL, Solid malignancy	ALX148 monotherapy, ALX148 plus pembrolizumab (Keytruda), ALX148 plus trastuzumab (Herceptin) or ALX148 plus rituximab (Rituxan)	USA, Korea
SRF231	1	Lymphoma, CLL, Solid malignancy	SRF231 monotherapy	USA

Source For Clinical Trial Information

https://clinicaltrials.gov

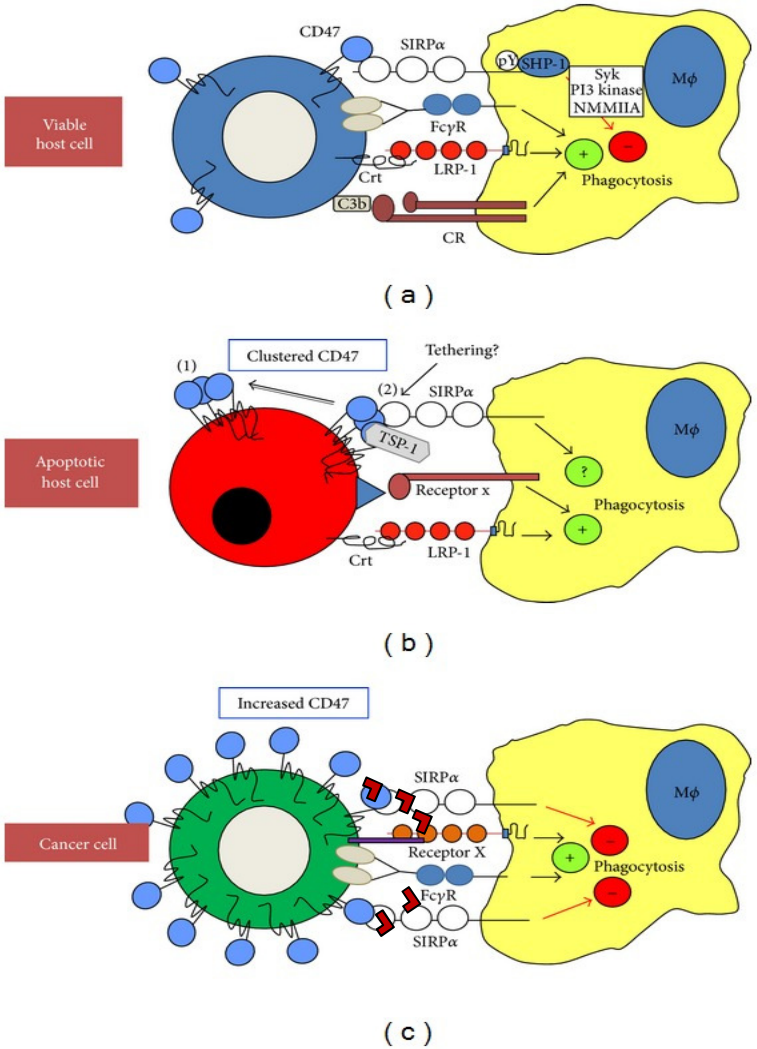
- Title and summary of trial
- Sponsor
- Investigational drug and/or drug combination/s
- Phase of trial
- Recruitment status
- Condition or disease treated
- Geographical locations
- Regional facilities participating
- Direct link to applicable reference publication/s
- End of trial results

Background On CD47



- 50 kDa multipass transmembrane glycoprotein
  - Member of the immunoglobulin superfamily (IgSF)
  - Expressed on virtually all tissue and cell types
- Innate immune checkpoint receptor
  - controls homeostatic phagocytosis
  - acts as important "marker of self"
- Protects transfused RBCs and platelets from rapid splenic clearance
- Binds SIRPα on macrophages ⟶ CD47/SIRPα ligation
  - delivers "do not eat me" signal to inhibit phagocytosis
- Up-regulation of CD47
  - protects hematopoietic stem cells by evading phagocytosis -good thing
  - leads to over expression in hematological and solid malignancies -bad thing
- Variation in molecule conformation
  - Conformation of CD47 may vary on RBCs
    - normal RBCs vs. sickle RBCs
    - new/fresh RBCs vs. aging RBCs
  - Senescent RBCs undergo CD47 conformational change- "CD47 Clustering"
    - "Eat me" switch ⟶ RBC clearance through apoptosis

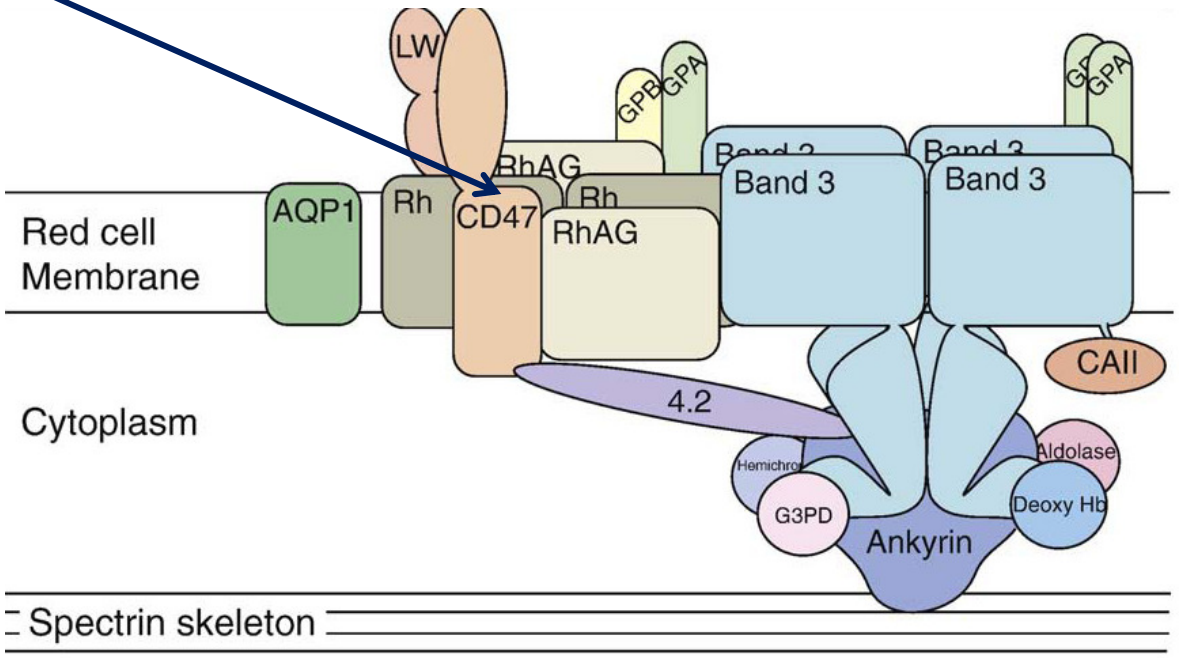
CD47 Expression



- **Normal CD47/SIRPα ligation**
  - evades phagocytosis
  - "do not eat me"
- **CD47 conformational change on senescent cell**
  - "CD47 Cluster"
  - CD47/TSP-1 ligation
  - CD47-induced apoptosis
- **CD47 up-regulation in tumor cells**
  - Block CD47/SRIPα ligation through:
    - mAb anti-CD47
    - Anti-CD47 fusion proteins
  - Triggers "Dinner Bell" for macrophages
    - "Eat me"
    - T-cell activation
    - NK-cell activation

Per-Arne Oldenborg, "CD47: A Cell Surface Glycoprotein Which Regulates Multiple Functions of Hematopoietic Cells in Health and Disease," *ISRN Hematology*, vol. 2013, Article ID 614619, 19 pages, 2013.

CD47 on human RBC membrane



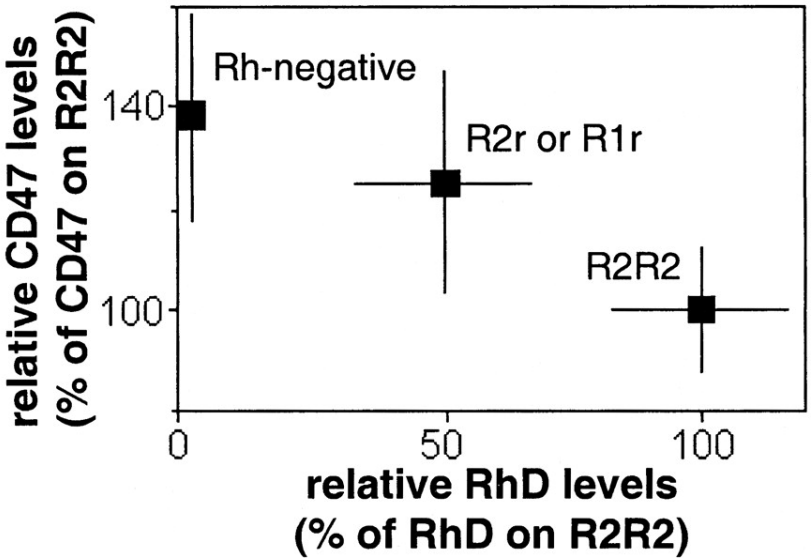
Part of the Rh complex

From Bruce, LJ; Blood Cells Mol Dis. 2009

- RhD & RhCE
- RhAG
- GPB
- LW
- CD47

CD47 Is Highly Expressed On RBCs

- **Expression varies by Rh phenotype**
  - **Rh negative – have highest expression**
  - **Rh positive, especially R2R2, have lower expression**



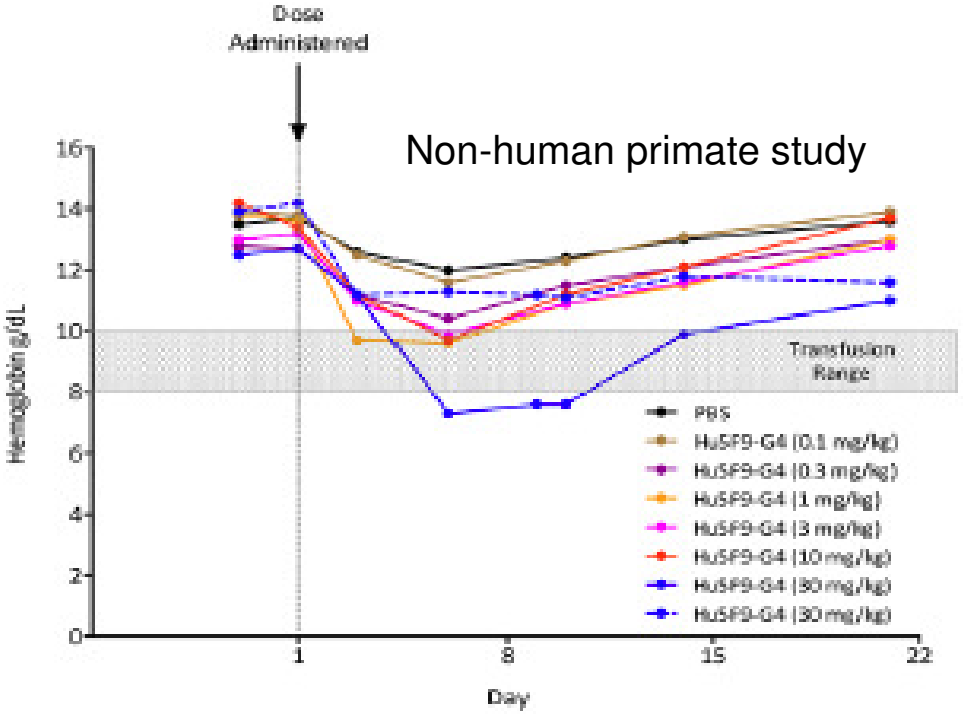
- CD47 levels related to Rhce in the membrane
  - RhD negative – (ce/ce) have more Rhce

Noel-Dahl K, Parthasarathy R, Westhoff CM, Layton DM, Discher DE, *Blood* 103: 1131-1136, 2004
Protein 4.2 is critical to CD47-membrane skeleton attachment in the human red cell..

Hu5F9-G4 Anti-CD47 Treatment

Anemia anticipated

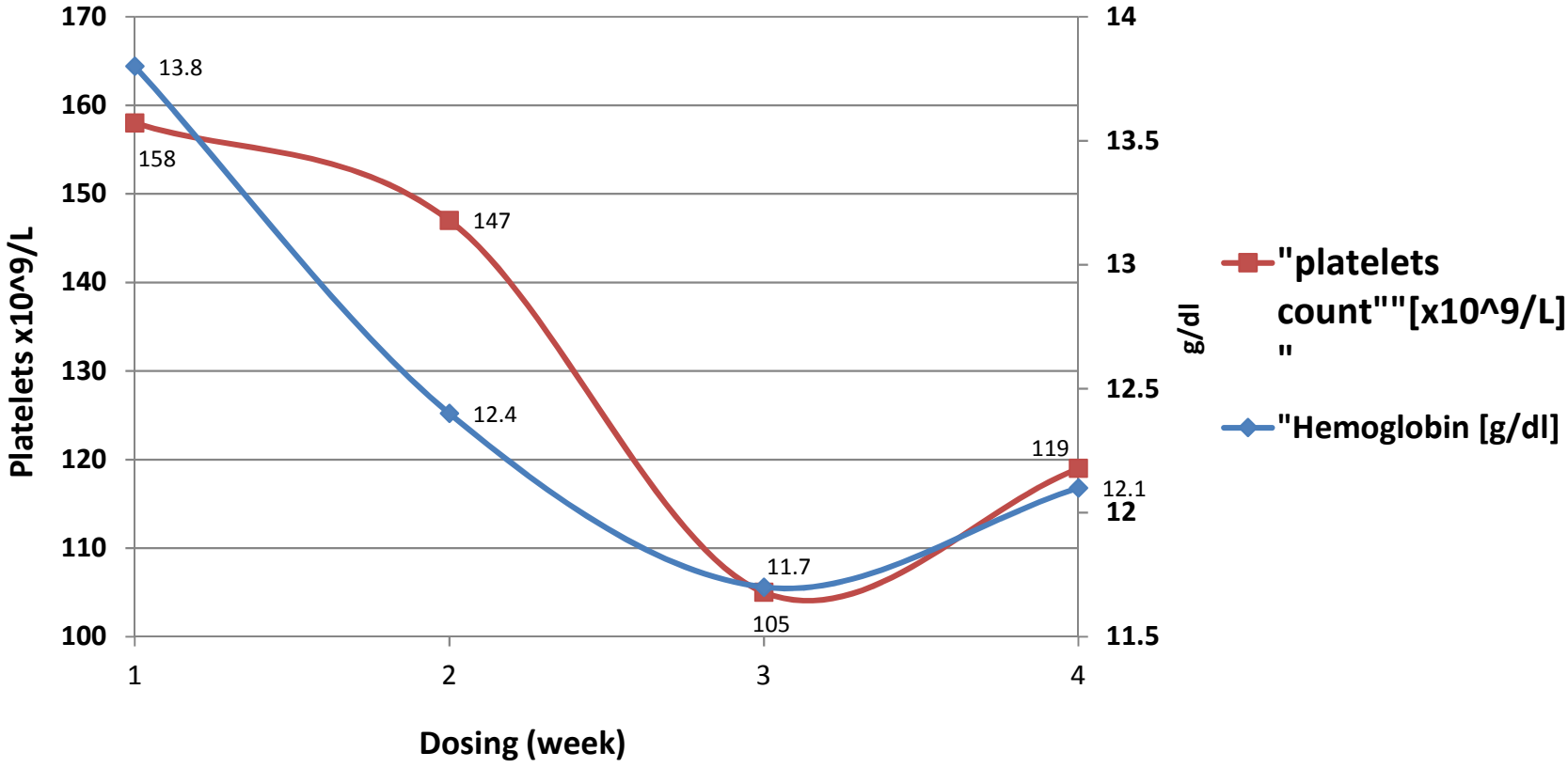
- reticulocytosis
- nadir of anemia on day 5-7
- anemia grade is dose dependent
- transient – resolves by day 25



Liu J, Wang L, Zhao F, Tseng S, Narayanan C, Shura L, et al. Pre-Clinical development of a humanized anti-CD47 antibody with anti-cancer therapeutic potential. PLoS ONE 2015;10:e0137345.

Hu5F9-G4 Anti-CD47 Treatment

Drop in hemoglobin values and platelet count



CD47 also highly expressed on platelets

Blood Bank Interference With Hu5F9-G4

- **Plasma reactivity - as soon as 1 hr post infusion**
- **Reactivity in all phases and by all methods: Gel, PEG, LISS, Albumin, Solid-Phase**
  - 3-4+ initial spin ⟶ IgM-like
  - 4+ in IAT
  - Carry-over agglutination from IS observed
  - Stronger reactivity with D– (rr) RBCs compared to D+
  - –D– RBCs weak
  - Rh_{null} RBCs weakest/negative
  - Negative auto control tests
- **Treated RBCs - reactivity remains with papain, ficin, trypsin, α-chymotrypsin, 0.2M DTT or W.A.R.M**

- **Interference in ABO typing**
  - Reverse/back type strongly reactive with A and B cells
  - Spontaneous agglutination in front/forward typing may be observed
  - Dependent on timing, dose and circulating plasma drug concentration

Blood Bank Interference With Hu5F9-G4

Negative DAT and auto control ⟶ likely false neg due to blocking

- **Eluate: 4+ pan-reactivity**
  - Indicates the anti-CD47 coating patient cells is causing steric interference or blocking in DAT and auto control tests

- **<u>Mitigation of plasma interference in the IAT</u>**
  - **Use of Immucor Gamma-clone anti-IgG (lacks IgG4)**
    - *Neat plasma– microscopic reactivity or negative*
      - 1+ reactivity observed in one recent case!
  - 4X **<u>allo</u>** adsorption with D– (rr) enzyme treated cells
  - 4X adsorption with platelets ⟶ pooled platelets or Immucor HPC
    - Degree of success dependent on timing, dosage and circulating plasma drug concentration…
  - PEG adsorption – precipitates anti-CD47 plasma!

Hu5F9-G4 Conclusions

- Plasma containing Hu5F9-G4 complicates serological testing
- Reactivity was observed as soon as 1 hour post infusion
- It interferes with routine pre-transfusion testing
  - Antibody screening/identification and crossmatch
  - ABO, Rh and extended antigen typing
- High levels of CD47 on RBCs results in agglutination at IS mimicking cold-reactive IgM antibodies
- Reactivity in all phases and all methods
- Plasma reactivity IAT titer ≥ 256 using Ortho anti-IgG
- CD47 not cleaved from RBCs by papain/ficin, trypsin, α-chymotrypsin, 0.2M DTT or W.A.R.M
  - Thus treatment does not mitigate interference

Hu5F9-G4 Conclusions Continued:

- Numerous adsorptions with either RBCs or pooled single donor platelets are required to remove anti-CD47 reactivity in plasma
  - **Efficacy of adsorption procedures related to circulating anti-CD47 concentration and date of last infusion**
- Use of Immucor Gamma-clone anti-IgG, which lacks IgG4, *may* mitigate interference in the IAT
  - However microscopic (or weak) reactivity may be observed
- DATs and auto control tests often negative due to blocking by anti-CD47
  - However, pan-reactive eluates may be obtained
    - Microscopic (or weak) reactivity using Immucor anti-IgG
    - Strong reactivity using Ortho anti-IgG (total IgG)

Future CD47 Therapeutics In Development

- **Generation of bispecific antibody variants**
  - **"BsAb"**
  - High-affinity to CD47
  - Co-targeting agents
  - Incorporated into a single molecule

→ Currently in pipeline: **anti-CD47 and CD20- "SIRPabody"**

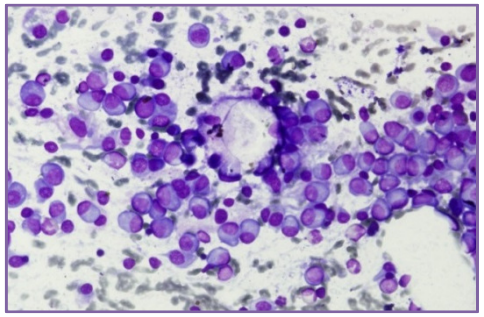
  - Functions as CD47 antagonist
    - Delivers highly effective blockade of CD47-SIRPα interaction
    - Binds minimally to CD47 molecule alone
    - Mitigates the toxic "antigen sink" caused by other anti-CD47 agents

Flashback Friday – except on a Wednesday!

CD38

Background On Daratumumab: Darzalex™ Anti-CD38

- **To treat multiple myeloma**
  - U.S. – approved for clinical trials in November 2015
    - Initially for patients relapsed with ≥3 prior therapies
    - Human IgG1-κ
    - Today much broader use
    - Combinational therapy "drug cocktails"
      - elotuzumab anti-SLAMF7
      - bortezomib, VELCADE™



Plasma Cells: High level of CD38

- **Recent FDA approval as frontline treatment for MM**

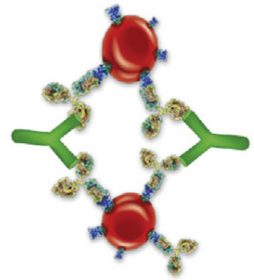
  - **Recent clinical trials**
    - Acute Lymphoblastic Leukemia (ALL)
    - Amyloidosis
    - Hodgkin & Non-Hodgkin Lymphoma
    - Acute Myeloid Leukemia (AML)
    - Chronic Lymphocytic Leukemia (CLL)

  currently emerging!

  - **Pipeline clinical trials**
    - Breast, lung, colon cancers and virus-associated tumors
    - Waldenström macroglobulinemia (Waldenström's)

Changing landscape of anti-CD38 therapeutics

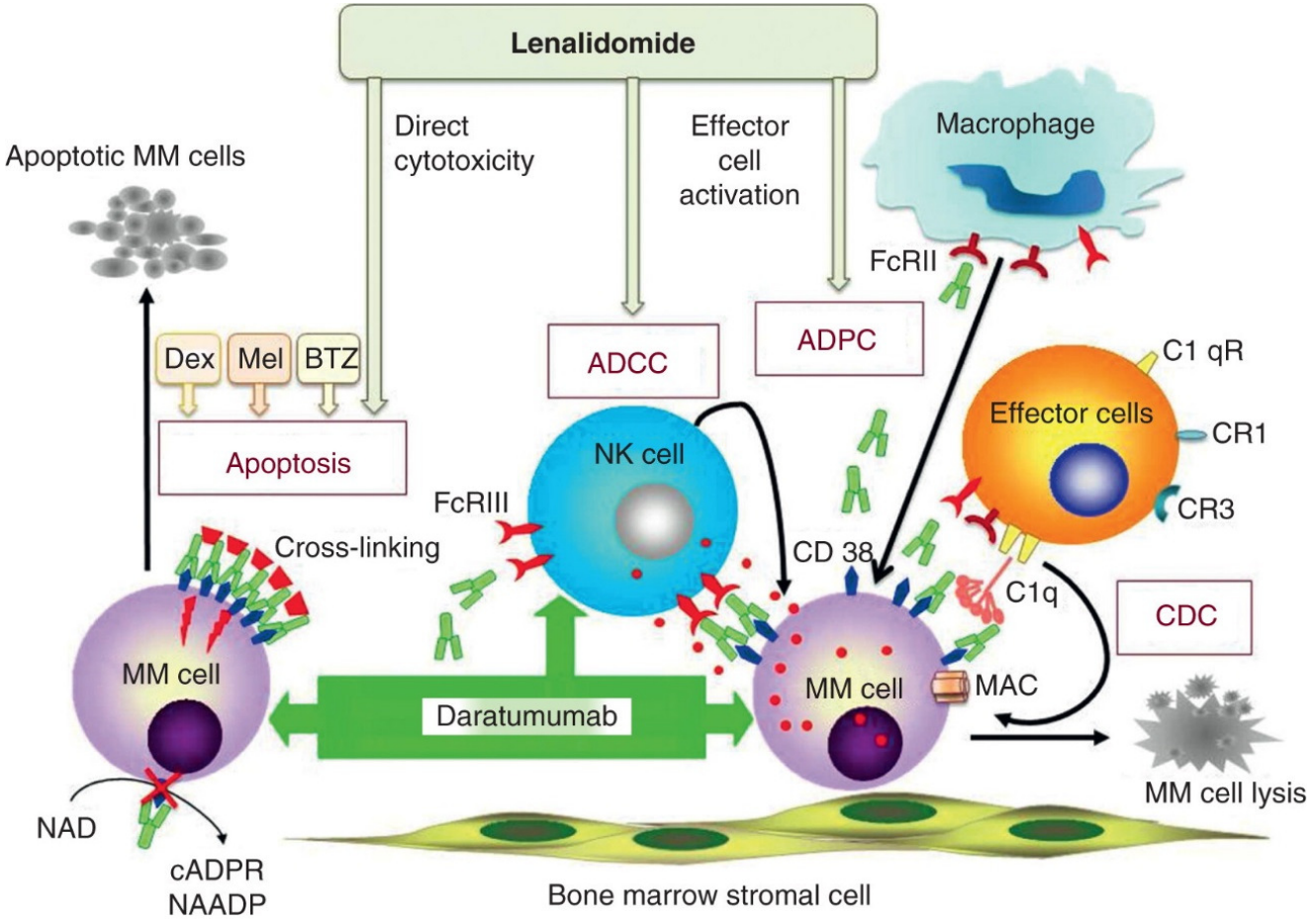
Agent	Developer	Description	Binds to RBCs
Isatuximab (SAR650984)	Sanofi-Aventis	Humanized IgG1-κ; chimeric	Yes →
MOR202	MorphoSys	Human IgG1-λ	Yes →
TAK-079 (Ab79)	Takeda	Human IgG1-λ	Pre-clinical
MT-4019	Molecular Templates	CD38-targeting engineered toxin body (ETB)	Pre-clinical



- Each bind to unique epitope on CD38
- May be used as single agents or in combination therapies
- Current FDA clinical trials
  - Isatuximab
    - MM, NHL, AML, ALL, CLL
    - USA, Europe and Asia
  - MOR202
    - Relapsed/Refractory Multiple Myeloma
    - Europe
- Future treatment
  - SLE, (other) solid tumor, management of autoimmune and allergic diseases

Anti-CD38 Mechanisms Of Action

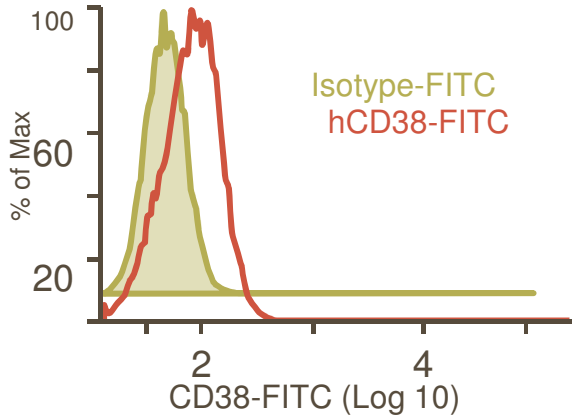
- Apoptosis /growth arrest
- Antibody-dependent cellular cytotoxicity (ADCC)
- Antibody-dependent cellular phagocytosis (ADCP)
- Complement-dependent cytotoxicity (CDC)



Jacob P Laubach; Yu-Tzu Tai; Paul G Richardson; Kenneth C Anderson; *Expert Opinion on Investigational Drugs* 2014, 23, 445-452.

DARA (Daratumumab;Darzalex™) Anti-CD38

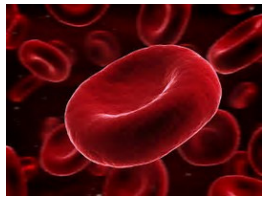
Chapuy CI et al.. *Transfusion.* 2015;55:1545-1554.



- **CD38 also expressed on RBCs**
  - **Very low levels**
  - Some variation has been observed
    - Fy(a–b–) RBCs
- **Excess anti-CD38 circulating free in patient plasma causes:**
  - positive antibody screen in **IAT**
  - positive crossmatch tests in **IAT**
    - Micro to 1- 2+ reactive depending on method
- **Does not interfere with ABO forward/reverse, Rh or antigen typing**
- **DAT may be negative or weakly positive**
  - DAT+ eluates may be negative or contain anti-CD38

DARA (Daratumumab;Darzalex™) anti-CD38

- **Why are patient's DAT and auto control tests non-reactive or only very weakly reactive?**
- **But panel and donor cells 1-2+ reactive?**



Drug induces loss of CD38 on patient's own RBCs

- **DAT+ immediately following DARA infusion**
  (no detectable RBC hemolysis)
- **Decrease of CD38 on patient RBCs within 6 hours**
- **CD38 undetectable in 1 week**
  - reversible - CD38 again detectable on RBCs 6 months after discontinuing drug
- **Loss of target antigen with administration of specific antibody**
  - Trogocytosis- antigenic modulation--"Downregulation"
  - Antigen "shedding"
  - Occurs through an Fcγ receptor dependent pathway

Approaches To Mitigate Anti-CD38 Interference In Plasma

- **Not successful**
  - **Multiple rounds of adsorptions with autologous or allogeneic RBCs**
    - **Using untreated, ZZAP, W.A.R.M or enzyme treated cells**
- **Successful**
  - ***Neutralize the drug antibody in patient plasma**
    - **with soluble recombinant CD38 protein- sCD38**
    - **with mouse-anti-Dara antibody- anti-idiotype**
  - **Treat TEST RBCs to remove CD38**
    - **0.2M DTT**
    - **Trypsin**
  - **Use panel cells that have undetectable CD38 for antibody ID**
    - **Dominant type Lu(a–b–), *In(Lu)* RBCs**
    - **Serological CD38-negative RBCs (DARA RBCs)**
    - **Cord RBCs**

*Chapuy CI et al. Resolving the daratumumab interference with blood compatibility testing. *Transfusion*. 2015;55:1545-1554

Challenges To DTT Or Trypsin Treating Test RBCs

- **DTT and Trypsin treated reagent RBCs not commercially available**
  - Must treat reagent RBCs in the blood bank laboratory
  - Write procedures
  - Train staff
  - Validation
  - **QC to confirm performance**
    - positive and negative controls
  - **Expiration of treated cells?**
    - establish laboratory storage limits
  - **Some clinically significant antigens also destroyed!**

Summary of anti-CD38 mitigation strategies

Method	Challenges	Antibodies missed
Plasma		
Anti-idiotype	Not readily available	None
Soluble CD38	Very expensive	None
Test RBCs		
0.2M DTT	Validation, training, QC, storage expiration	KEL, DO, IN, LW, JMH, KN, LU, YT, CROM, MER2
Trypsin		DO, MN, LU, Ge, Ch/Rg, XG, JMH, MER2, KN, IN
Cord cells	Need extended typing	phenotype dependent P1, LU, AnWj, Le, Ch/Rg, Vel, Fy3, YT, JMH, Xg^a, KN; some antigens only weakly expressed
In(Lu) cells	Rare/Uncommon	phenotype dependent
DARA-RBCs	Not readily available to blood banks, need extended typing	None when covering with selected phenotypes

Common Practice:

- Use DTT treated cells AND transfuse K– RBCs
- Use Trypsin treated cells to rule out Kell, LW, Yt (most often used in our lab)

Pipeline Anti-CD38 Mitigation Agents

Method	Manufacturer	Reference
Plasma		
Recombinant CD38 (sCD38)	Medion Grifols Diagnostics/Grifols Diagnostic Solutions	Binda M *et al*. Novel Recombinant CD38 For Use In Pretransfusion Diagnostics. *Vox Sanguinis* 2018;113,Issue S1, P-525
Test RBCs		
DaraEx (anti-CD38 antibody neutralizing agent)	Immusyn inno-train	Schneeweiss C *et al*. Daratumumab interference in red cell antibody detection: specific inhibition with DaraEx. *Transfusion Medicine and Hemotherapy* 2017; 44, Supplement 1, P06-5

New Recently Described Mitigation Techniques

- Plasma adsorptions with cell lines that highly express CD38
  - Tremblay T *et al*. New Approaches To Eliminate CD38 Monoclonal Antibodies Related Interference in Pre-Transfusion Testing. *Vox Sanguinis* 2018;113,Issue S1, 3A-S02-03
- Modification of protocol for DTT treatment of test RBCs
  - Lone Akhtar *et al*. A Modified Dithiothreitol Protocol For Eliminating Daratumumab Interference. *Vox Sanguinis* 2018;113,Issue S1, 3A-S02-03

Comparison Of Anti-CD38 And Anti-CD47 Interference

	CD38	CD47
RBC expression	LOW	HIGH
Interference	Anti-CD38	Anti-CD47*
ABO typing	No	Yes (in reverse)
RhD typing	No	possible
Other antigens	No	possible

- Depends on *specific drug**, dosage and timing

Comparison of anti-CD38 and anti-CD47 interference

	CD38	CD47*
RBC expression	**LOW**	**HIGH**
Interference*	**Anti-CD38**	**Anti-CD47***
ABO typing	**No**	**Yes (reverse)**
RhD typing	**No**	**possible**
Other antigens	**No**	**possible**
Antibody screen	IAT only (micro to 1-2+)	All phases (3-4+)**
Crossmatch	IAT only (micro to 1-2+)	All phases (3-4+)**
DAT/auto control	neg (wk+)	false negative
Eluate	neg (wk+ anti-CD38)	strong panreactive (3-4+)
Adsorptions	No	4X RBCs or platelets

- Depends on *specific drug**, dosage and timing

** Negative to weak IAT with anti-IgG lacking IgG4 (Gamma-clone)

Summary anti-CD38 and anti-CD47 mitigation

Mitigation	CD38	CD47
	*0.2M DTT treat test RBCs	** Gamma-clone anti- IgG may not detect
	*Trypsin treat test RBCs	Alloadsorptions with RBCs or platelets may remove
	Inhibition substances	
	Serological CD38-negative test RBCs	

CD38 –* DTT and trypsin destroy significant antigens

- Inhibition substances are costly
- *To date no clear consensus reached on mitigation recommendation*

CD47 – Interference will be drug specific

**Hu5F9-G4 – IgG4 to CD47

**CC-9002 – IgG4 to CD47

Potentially low or no binding to RBCs – CD47/SIRPα antagonist

- TTI-621 – IgG1 targeting CD47 binding domain of SIRPα
- ALX148 – inactive Fc targeting binding domain of SIRPα

Summary Of Evolution In Monoclonal Antibody Therapies

- Single-agent mAbs
  - Darzalex™ monotherapy
- Drug cocktails
  - mAb plus Imid
    - E.g. Daratumumab plus Velcade™
- Bispecific antibodies- bsAb
  - E.g. CD47/CD20-co-targeting
- High affinity soluble fusion proteins- FPs
- mAb-drug conjugates
  - Engineered Toxin Bodies (ETBs)
- What does the future hold???
  - Endless possibilities… so stay tuned!

Thank You !

New York Blood Center

Immunohematology and Genomics Laboratory