

SAR650984: A potent humanized anti-CD38 therapeutic antibody for hematologic malignancies, including multiple myeloma and non-Hodgkin's lymphoma

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Introduction

- **CD38 Function:**
 - **CD38 is a 45 kD type II transmembrane glycoprotein ¹**
 - **It contains NADase ectoenzyme activity and has been implicated in Ca²⁺ mobilization and signaling ^{2,3}**
 - **CD38^{-/-} mice are viable without histological or pathological abnormalities ⁴**
- **CD38 expression:**
 - **The CD38 antigen is expressed during the early stages of T- and B-lymphocyte differentiation, and during the final stages of maturation including differentiated plasma cells ⁵**
 - **It is not expressed on normal pluripotent early stem cells, which are defined as CD34⁺, CD38⁻ ⁶**

1 Ferrero 1999 J Leukoc Biol. 65:151-61; Review

2 Lee 1999 Biol. Chem. 380, 785–793; Review

3 Mehta 1996 FASEB J. 12:1408-17; Review

4 Cockayne 1998 Blood, 92:1324-1333

5 Konopleva 2000 Chem Immunol. 75:189-206; Review

6 Terstappen 1991 Blood 77:1218-27.

CD38 and hematologic malignancies

- CD38 is expressed in many hematologic malignancies:
 - Non-Hodgkin's lymphoma (NHL), including mantle cell lymphoma (MCL) ¹ and diffuse large B-cell lymphoma (DLBCL) ², 30-80%
 - Multiple myeloma (MM) ³, 80-100%
 - B chronic lymphocytic leukemia (B-CLL) ⁴, 20-25%
 - B and T acute lymphoblastic leukemia (B- and T-ALL) ⁵, 90-100%
 - Acute myeloid leukemia (AML) ⁶, 58%
- CD38 expression is correlated with disease progression and poor prognosis in B-CLL ⁷
- Increased number of circulating CD38⁺ plasma cells is an indicator of poor survival in MM ⁸

1 Angelopoulou, Eur J Haematol 2002;68:12-21

2 Schwonzen, Brit J Haematol 1993;83:232-239

3 Lin, Am J Clin Pathol 2004;121:482-488

4 Domingo-Domènech, Haematologica 2002;87:1021-1027

5 Koehler, Leukemia 1993;7(1):41-45

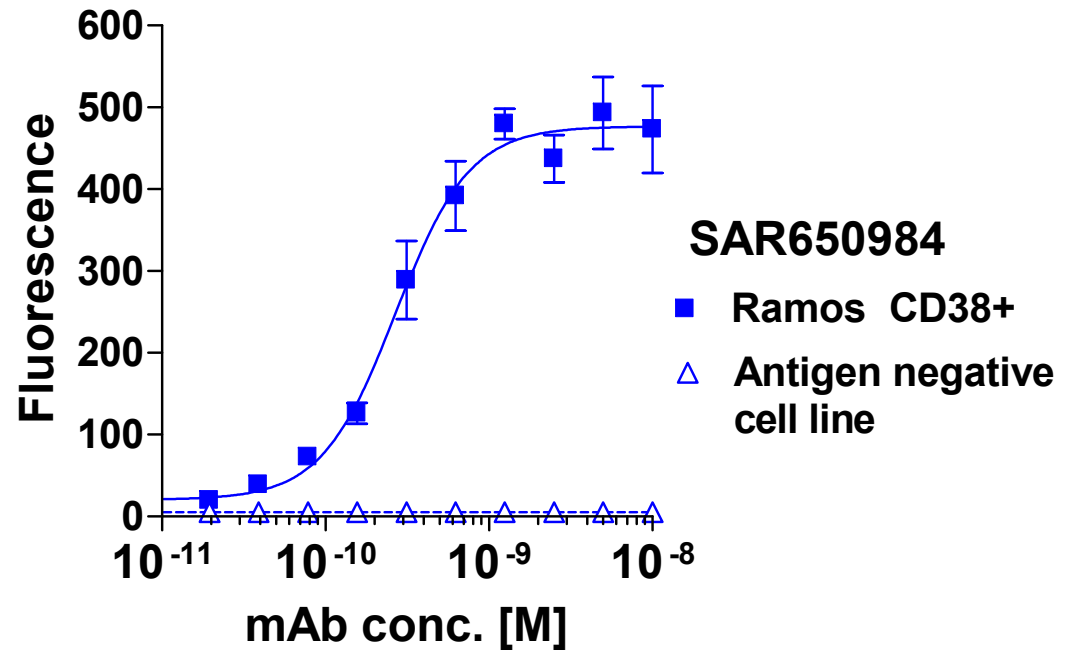
6 Keyhani, Leukemia Res 1999;24:153-159

7 Ghia, Blood. 2003;101:1262-1269

8 Nowakowski, Blood. 2005;106:2276-2279

SAR650984 antibody

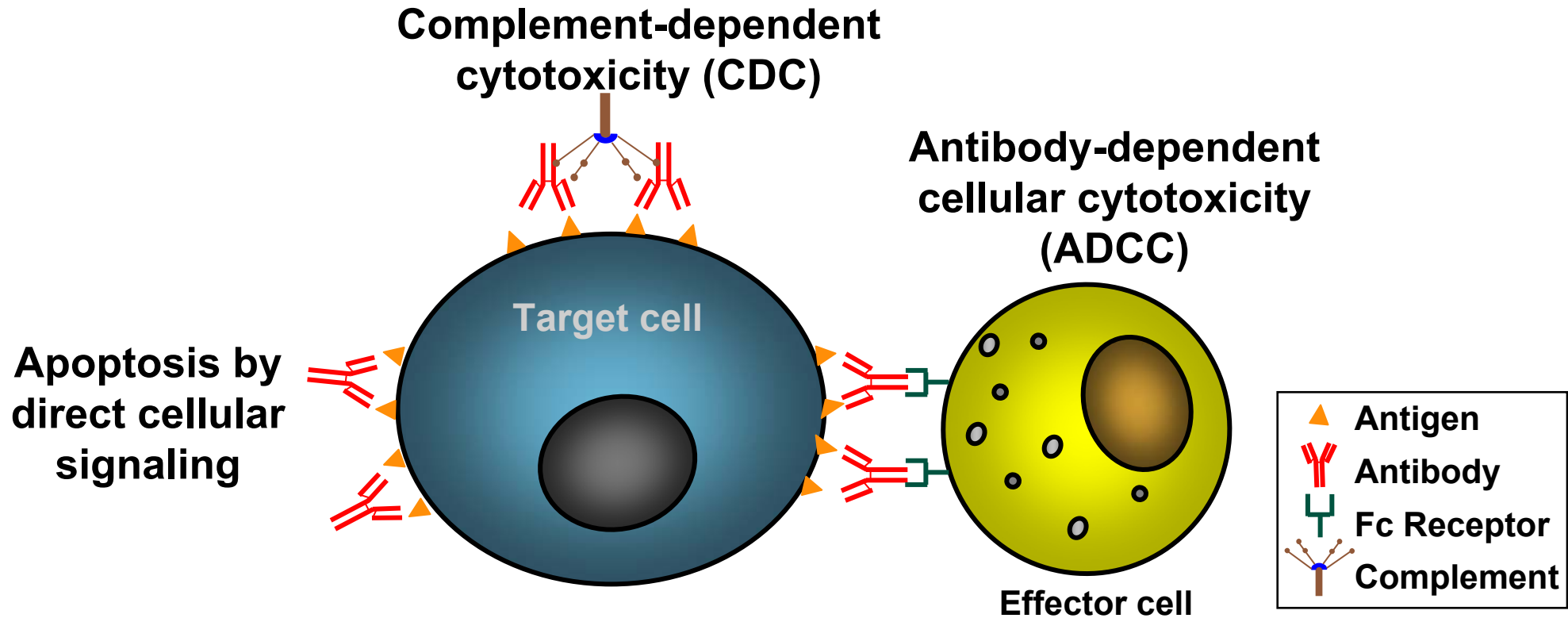
- SAR650984 is a humanized anti-CD38 antibody co-developed with sanofi-aventis
- Antibody was humanized to hulgG1 using ImmunoGen's resurfacing technology
 - Antibody contains fully human IgG1k constant regions
 - murine surface residues in variable regions replaced by corresponding human residues
- SAR650984 binds specifically and with high affinity to human CD38
- $K_D = 0.2$ nM



- Binding assay:
 - flow cytometry with AF488-labeled SAR650984 on antigen positive Ramos cells

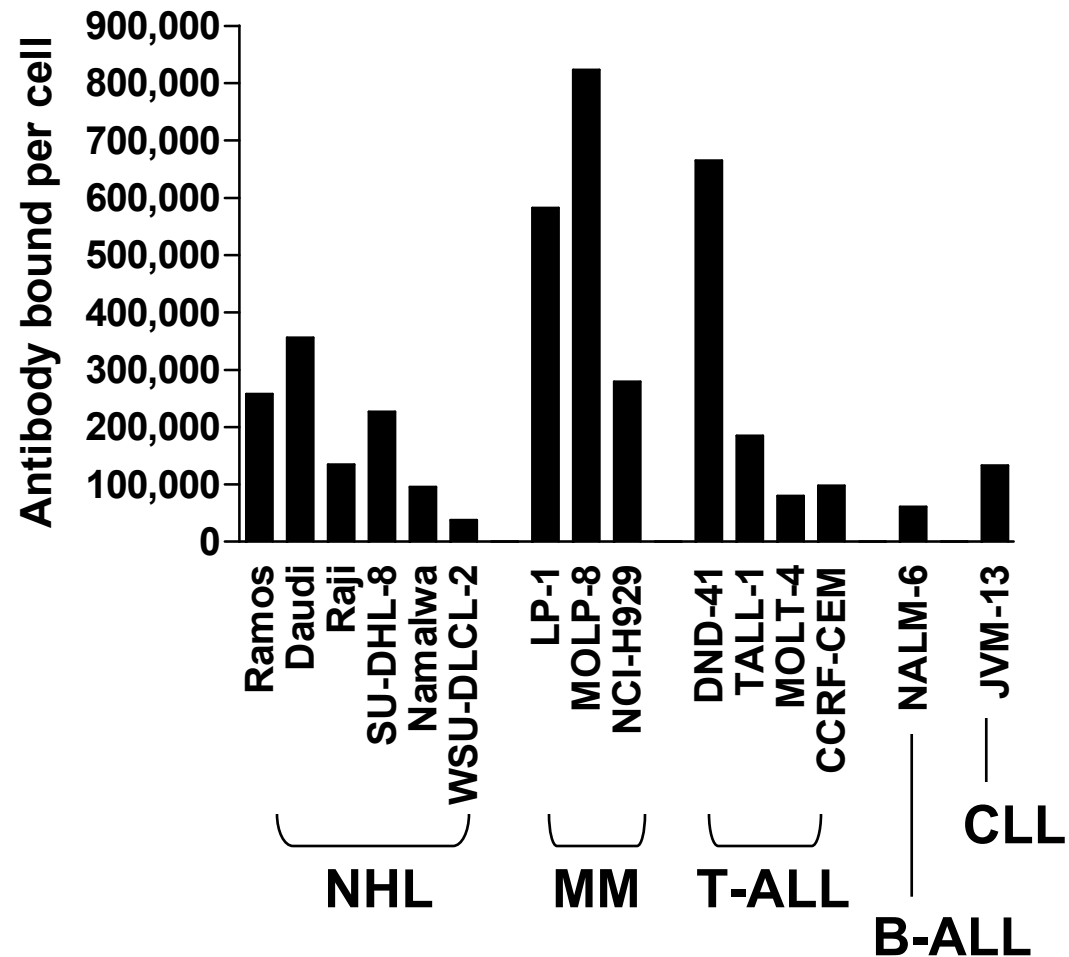
SAR650984 mechanism of action

- SAR650984 was selected for its potent *in vitro* activity through multiple mechanisms of action: apoptosis, complement-dependent cytotoxicity (CDC), and antibody-dependent cellular cytotoxicity (ADCC).



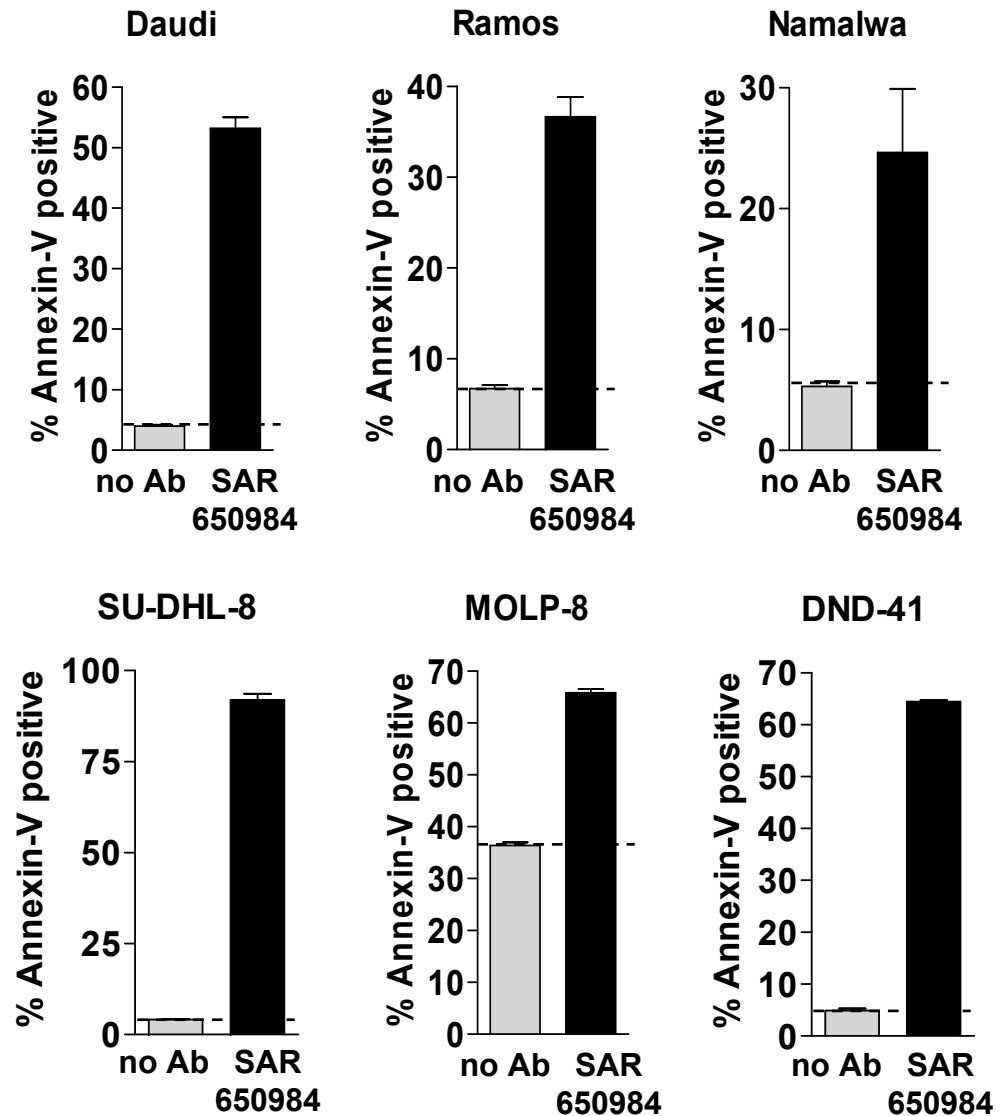
CD38 antigen expression on tumor cell lines

- CD38 expression levels were measured quantitatively
 - BD QuantiBRITE™
- ABC (Antibodies Bound per Cell) values: 60,000 - 823,000
- Cell lines derived from various hematologic malignancies were CD38-positive:
 - Highest expression in MM cell lines
 - CD38 expression detected in non-B-cell malignancies such as T-ALL



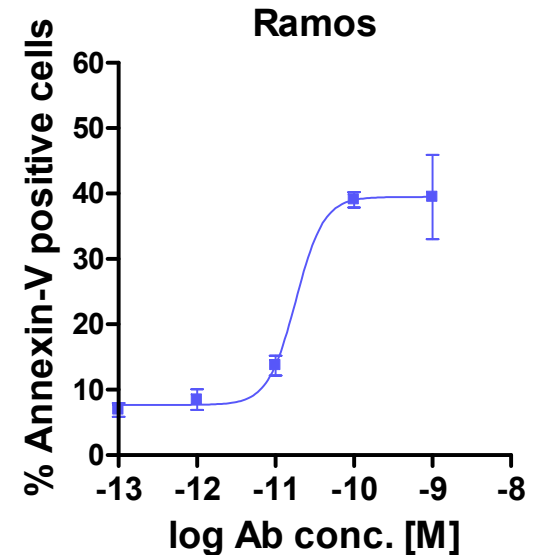
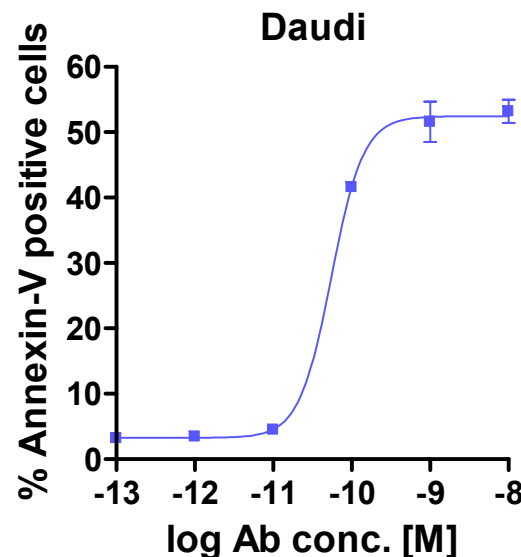
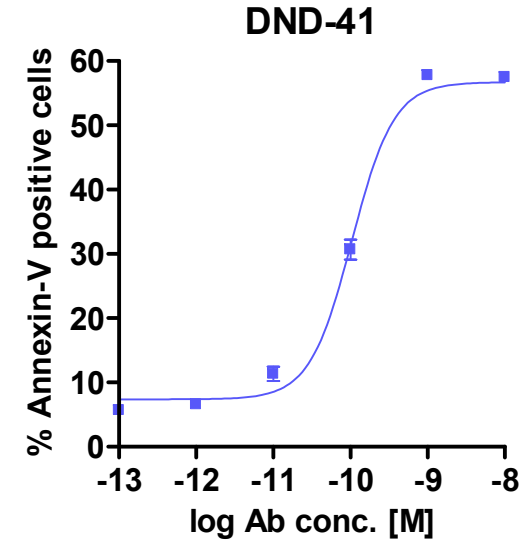
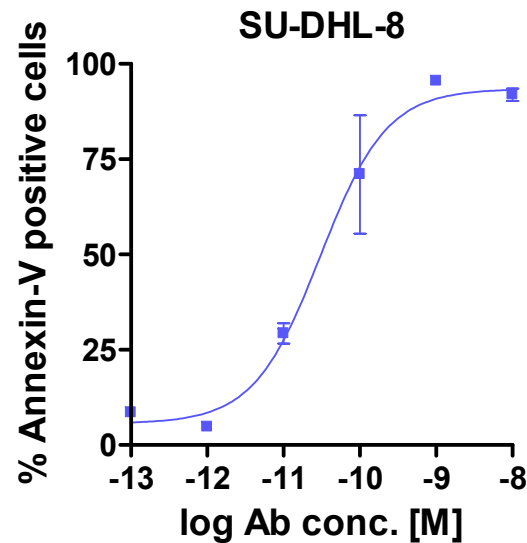
SAR650984 has strong pro-apoptotic activity

- SAR650984 showed pro-apoptotic activity against 7 of 15 cell lines tested:
 - Daudi, Ramos, SU-DHL-8, Raji, Namalwa, MOLP-8, and DND-41.
- Pro-apoptotic activity did not require cross-linking agents
- Maximum percentage of Annexin-V positive cells: 11% - 90%
- Apoptosis assay:
 - Incubated target cells and 1.5 µg/ml Ab for 20 hrs at 37°C followed by Annexin-V staining



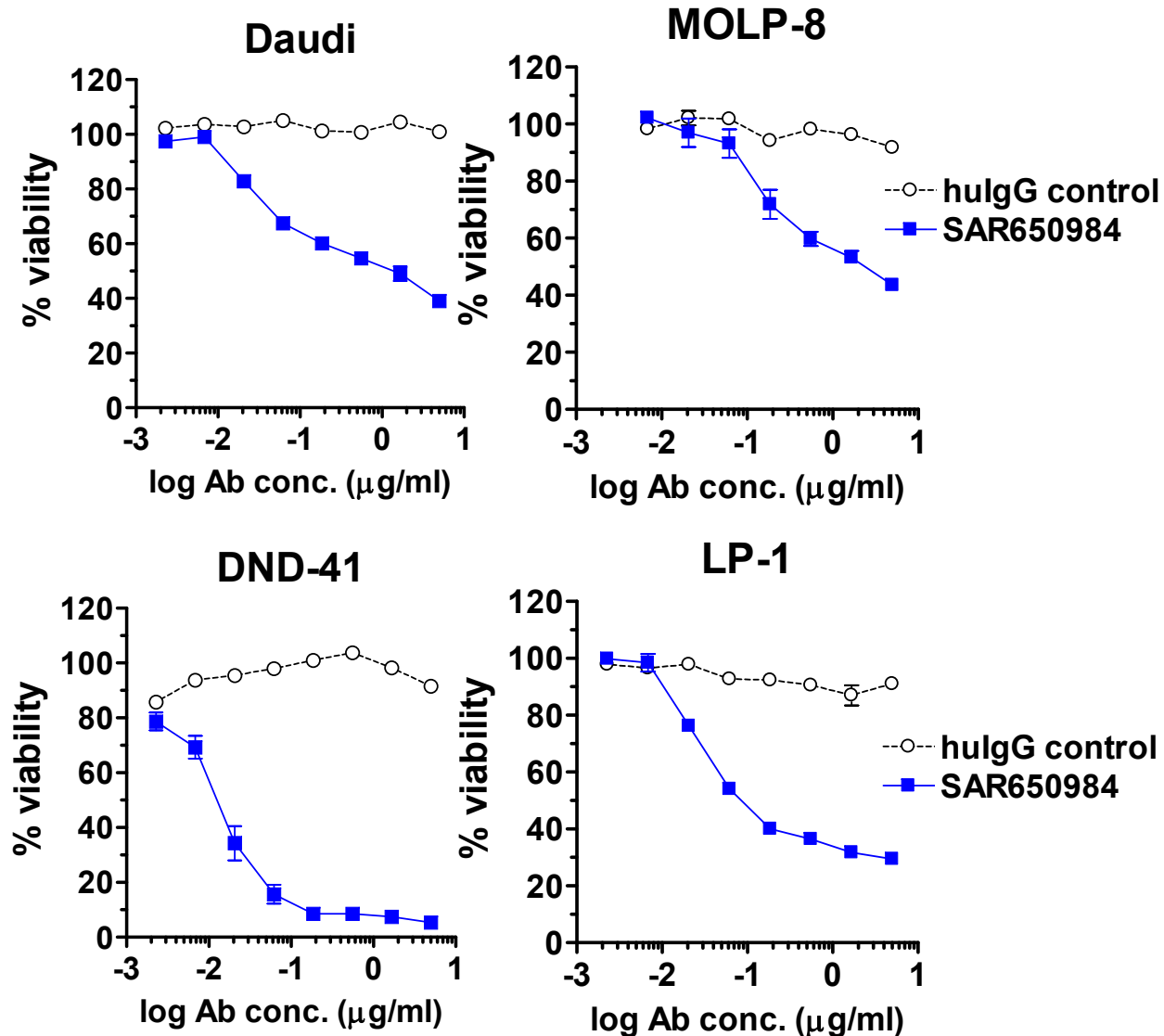
Pro-apoptotic activity of SAR650984 is dose-dependent

- SAR650984 pro-apoptotic activity was determined in a dose-response by Annexin-V staining on:
 - SU-DHL-8, DND-41, Daudi, Ramos and Namalwa
- Dose-dependent activity
- EC50 values: 0.02 - 0.2 nM
- Ab binding K_D : 0.2 nM



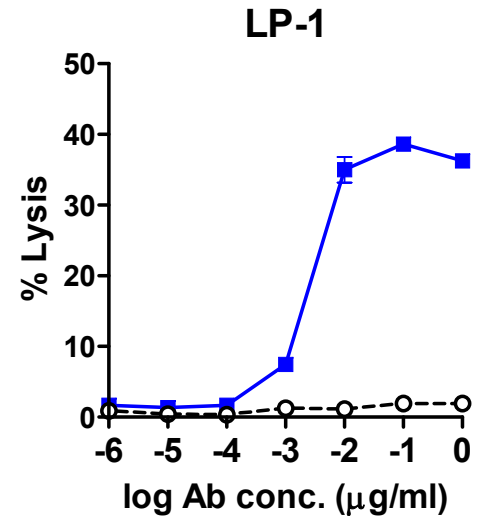
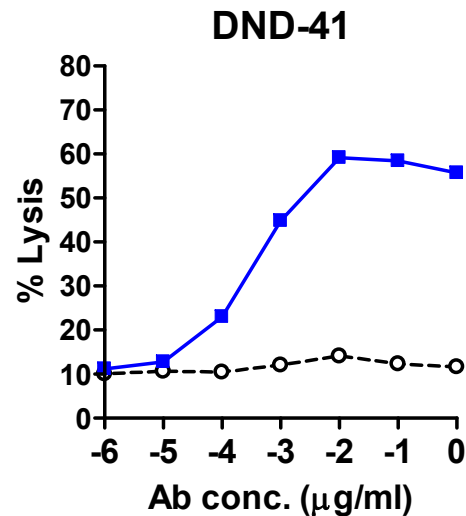
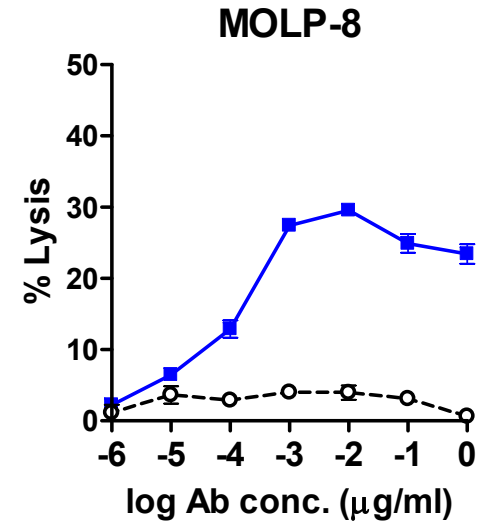
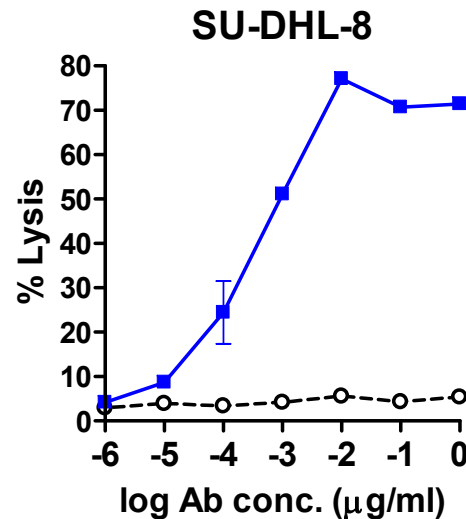
SAR650984 has potent CDC activity

- SAR650984 mediated strong complement-dependent cell lysis against 7 of 15 cell lines in the presence of human serum:
 - Daudi, Ramos, SU-DHL-8, Raji, MOLP-8, LP-1, and DND-41
- Maximum percentage of specific lysis: 22% - 95%
- EC50 values: 0.1 nM - 1.3 nM
- CDC assay:
 - Incubated cells with Ab and 5% human serum for 2 hrs at 37°C, Alamar Blue assay



SAR650984 has potent ADCC activity

- SAR650984 induced potent ADCC activity through human NK cells against all tumor cell lines tested
- Even cell lines with moderate CD38 expression were subject to ADCC activity mediated by SAR650984
- Maximum percentage of NK-mediated lysis: 23% - 100%
- EC50 values: 1 - 30 pM
- ADCC assay:
 - Incubated targets cell with Ab and purified human NK cells for 4hrs at 37°C, LDH release assay
 - Typical effector: target ratio = 3:1



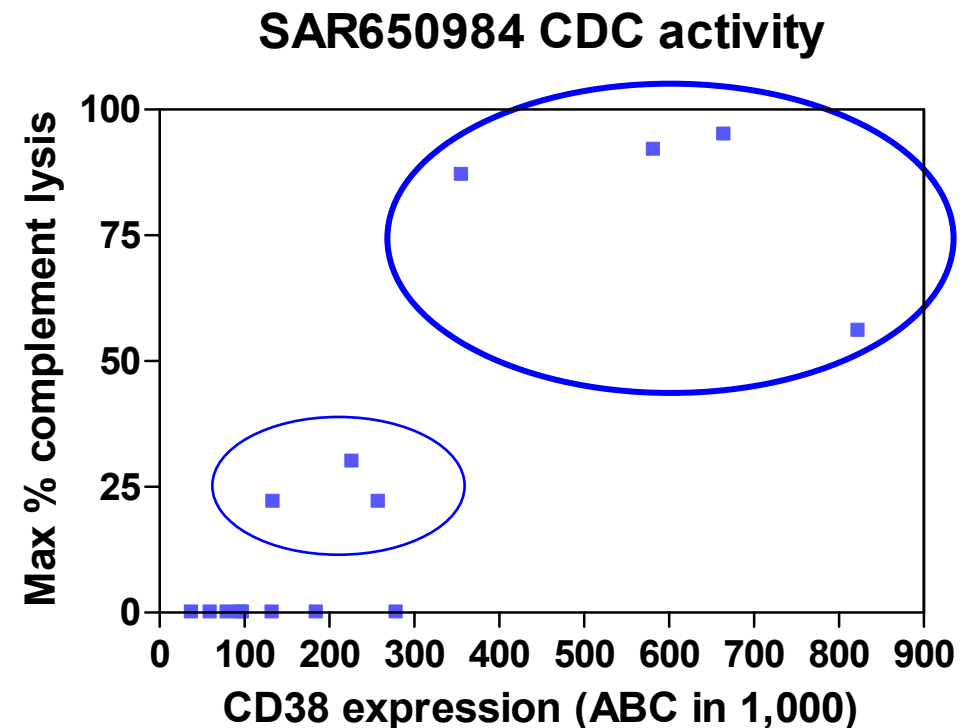
■ SAR650984
-○- hulG control

SAR650984 is active against a panel of cell lines *in vitro*

| Origin | Cell line | Antigen expression | Apoptotic activity | | ADCC activity | | CDC activity | |
|--------|-----------|--------------------|--------------------|---------------|---------------|-------------|--------------|-------------|
| | | ABC in 1,000 | EC50 | Max % Ann. V+ | EC50 | Max % lysis | EC50 | Max % lysis |
| NHL | Daudi | 356 | 0.2 nM | 63 | 1.7 pM | 83 | 0.26 nM | 87 |
| | Ramos | 258 | 0.02 nM | 30 | 4.3 pM | 90 | 0.1 nM | 22 |
| | SU-DHL-8 | 227 | 0.03 nM | 90 | 2.3 pM | 75 | 0.4 nM | 30 |
| | Raji | 134 | ND | 11 | 8.2 pM | 53 | 0.15 nM | 22 |
| | Namalwa | 95 | 0.02 nM | 25 | 3.1 pM | 46 | - | - |
| | WSU-DLCL2 | 38 | - | - | 2.7 pM | 41 | - | - |
| MM | MOLP-8 | 823 | ND | 27 | 1.1 pM | 39 | 1.3 nM | 56 |
| | LP-1 | 582 | - | - | 17 pM | 28 | 0.26 nM | 92 |
| | NCI-H929 | 279 | - | - | 30 pM | 23 | - | - |
| T-ALL | DND-41 | 665 | 0.1 nM | 62 | 3 pM | 47 | 0.11 nM | 95 |
| | TALL-1 | 185 | ND | 3 | 1.0 pM | 100 | - | - |
| | CCRF-CEM | 98 | - | - | 1.0 pM | 29 | - | - |
| | MOLT-4 | 80 | ND | 4 | 1.0 pM | 20 | - | - |
| B-ALL | NALM-6 | 60 | - | - | 10 pM | 37 | - | - |
| B-CLL | JVM-13 | 133 | ND | 3 | 27 pM | 17 | - | - |

Antigen density and CDC *in vitro* activity correlation

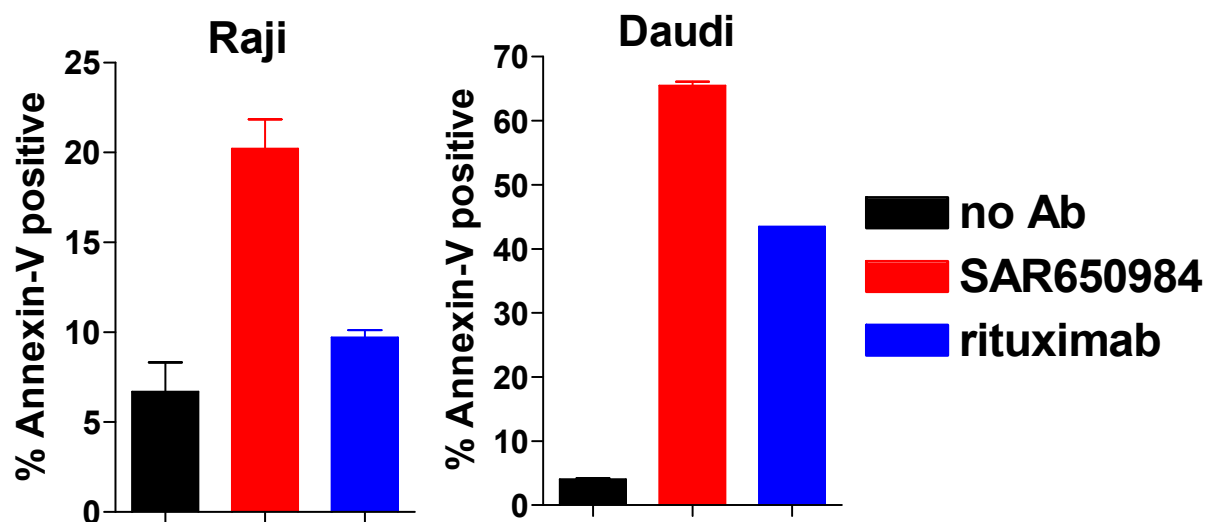
- SAR650984 CDC activity:
 - Most potent CDC activity on cell lines expressing the highest levels of CD38
 - Intermediate CDC activity against cell lines with moderate CD38 expression
- No correlation between the levels of antigen expression and apoptotic or ADCC activity could be obtained in this set of cell lines



SAR650984 showed a higher pro-apoptotic activity than rituximab in Raji and Daudi lymphoma cells

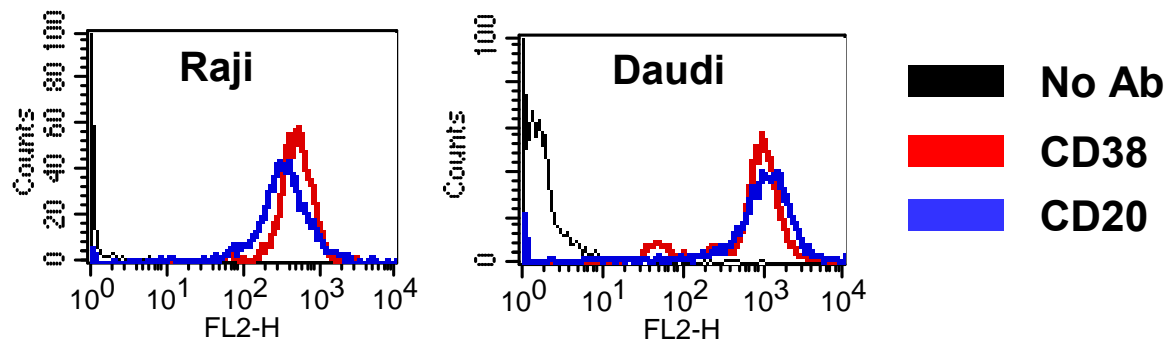
- In Raji cells SAR650984 treatment of resulted in an increase in apoptotic cells from 7% to 20% (vs. 10% in rituximab treated cells)
- In Daudi cells SAR650984 increased the percentage of apoptotic cells from 4% to 65%, (vs. 43% in rituximab treated cells).

Pro-apoptotic activity



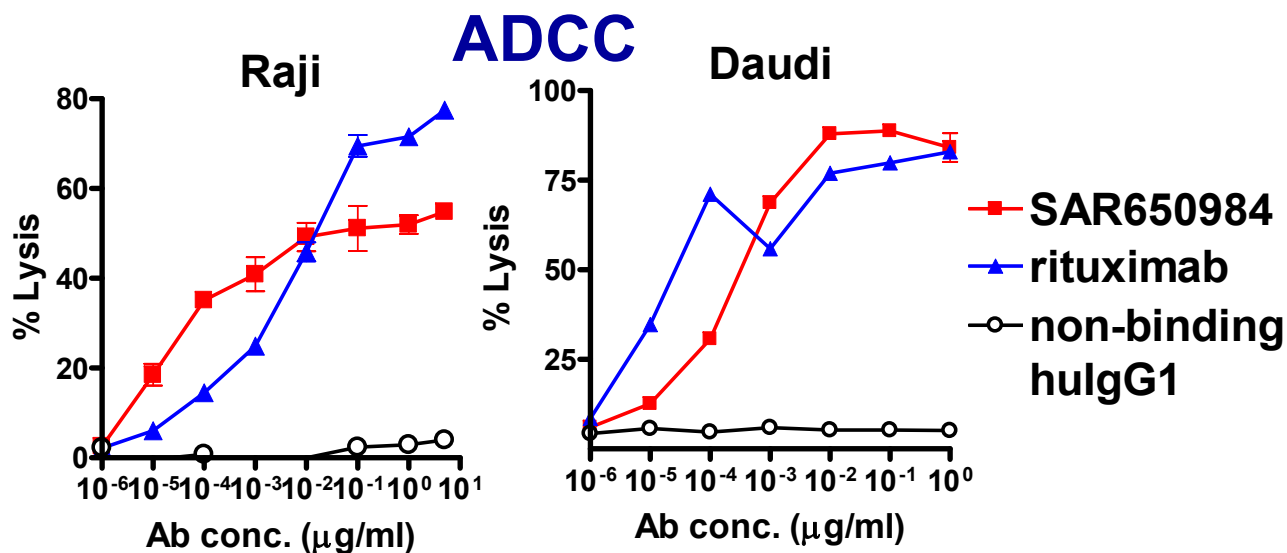
- Both Raji and Daudi cells express CD38 and CD20 at similar levels when compared by flow cytometry

Antigen expression: CD38 vs. CD20

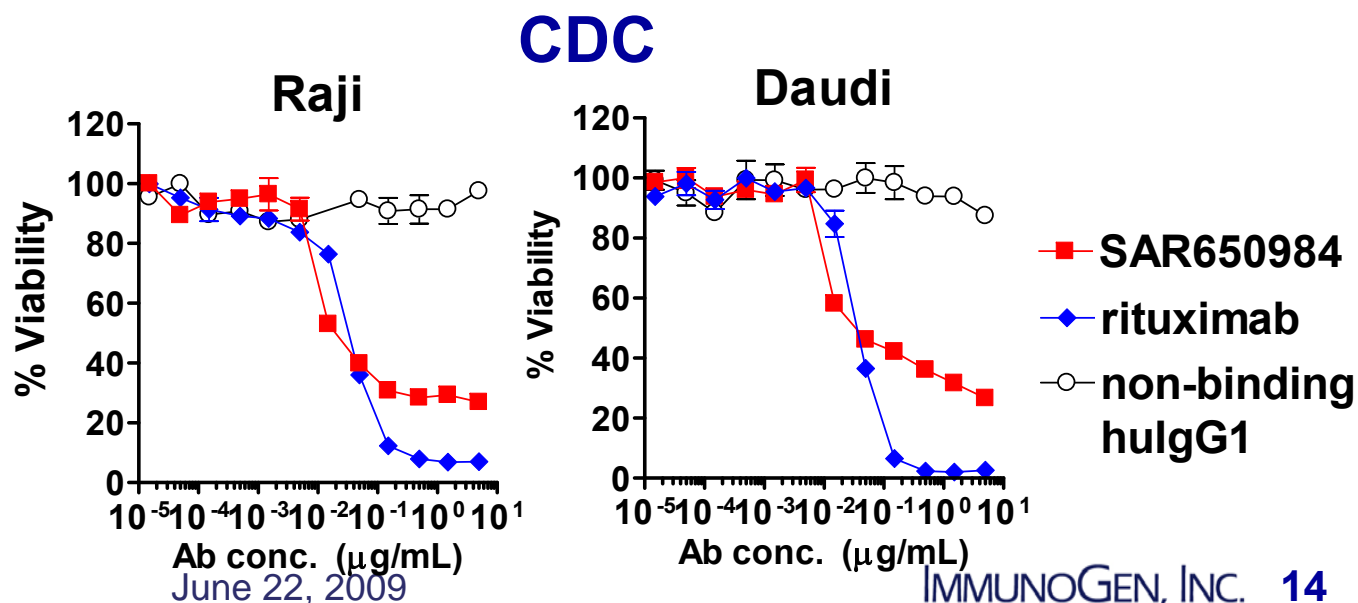


SAR650984 and rituximab showed similar ADCC and CDC activities in Raji and Daudi lymphoma cells

- ADCC: SAR650984 and rituximab were able to mediate a similar degree of specific cell lysis of Raji and Daudi cells

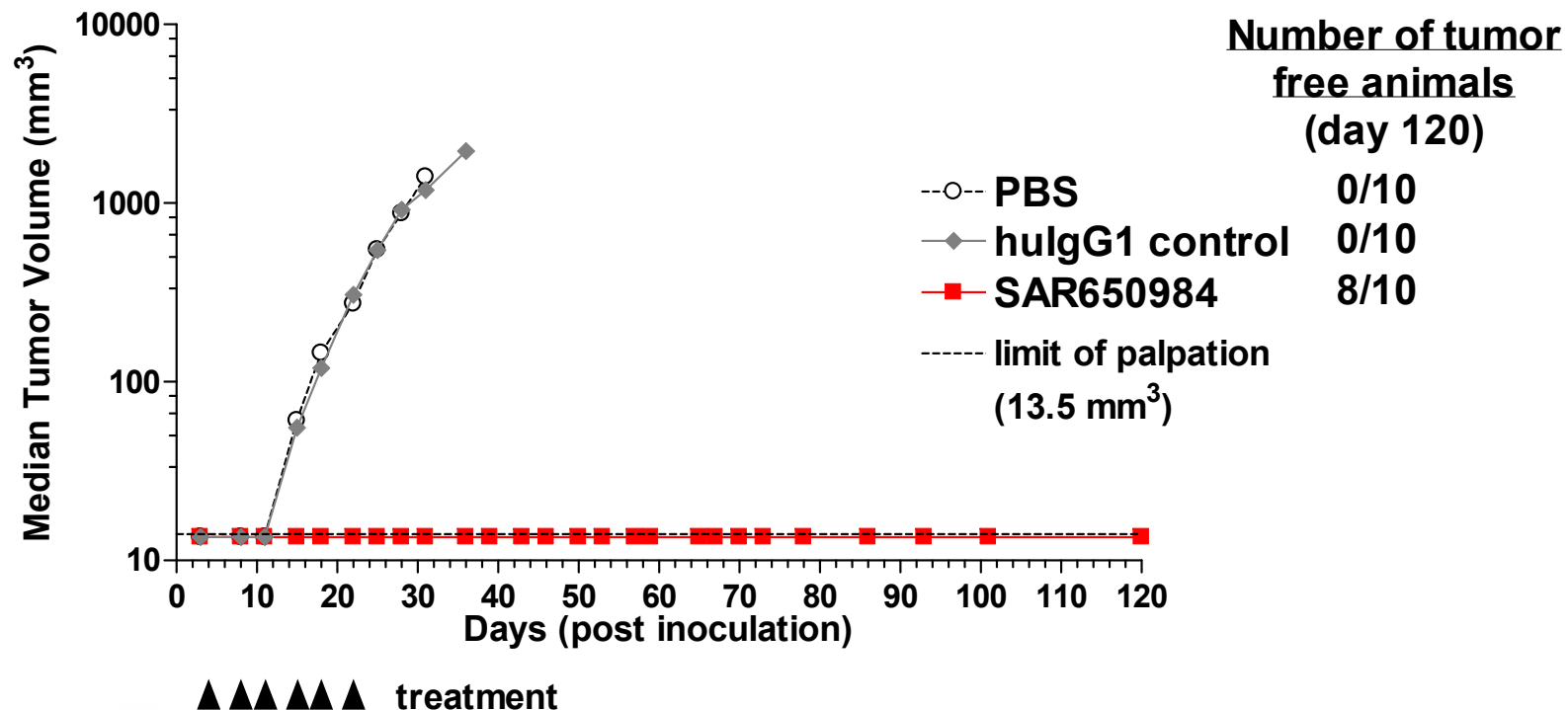


- CDC: SAR650984 and rituximab had a similar CDC activity against Raji and Daudi cells



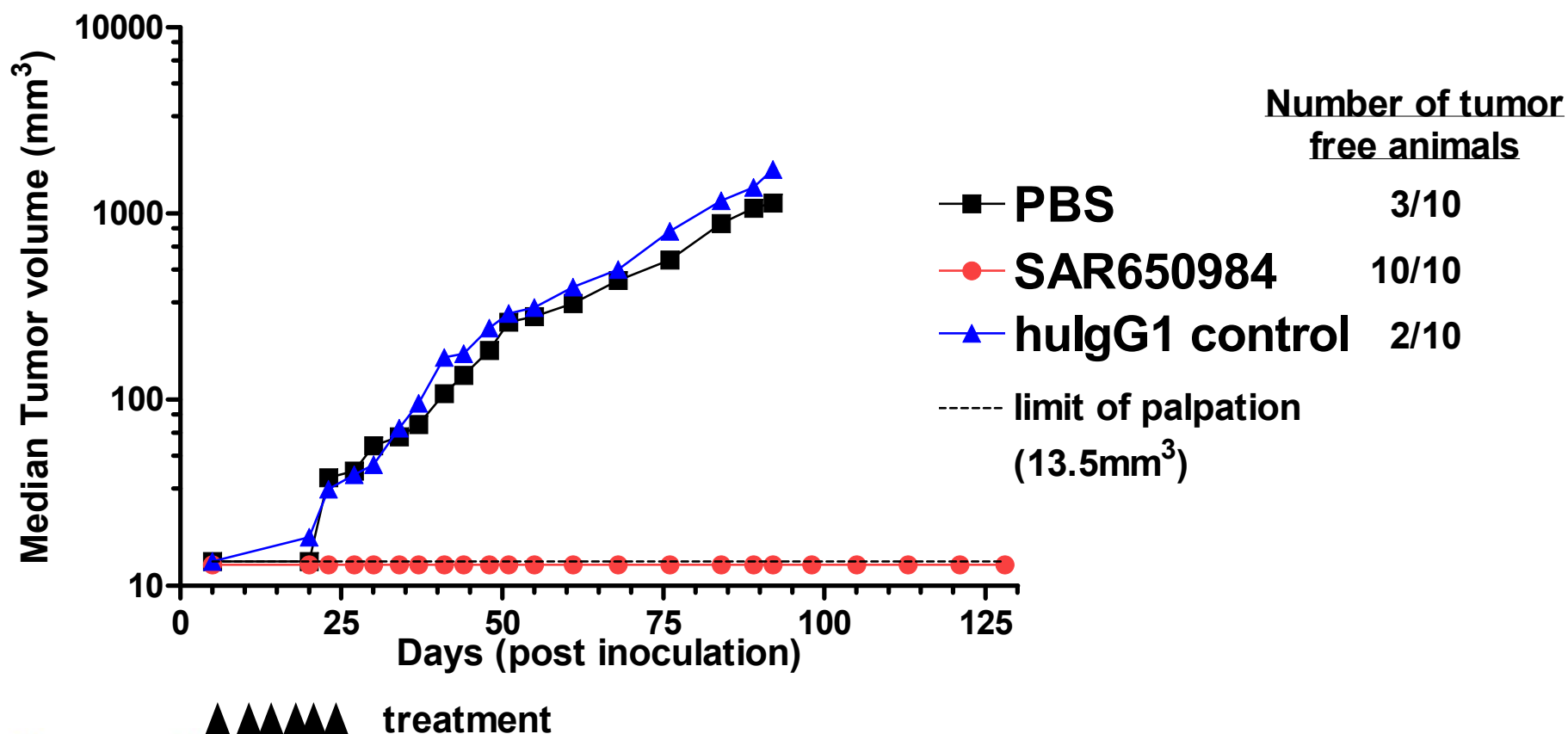
SAR650984 shows strong *in vivo* efficacy in a MOLP-8 multiple myeloma model

- Early stage Molp-8 multiple myeloma model (palpable tumors)
 - Schedule: 40 mg/kg of SAR650984 twice weekly for 3 weeks
 - 8 of 10 animals remained tumor free after SAR650984 treatment
- SAR650984 was active *in vitro* against this multiple myeloma cell line through three mechanisms of action



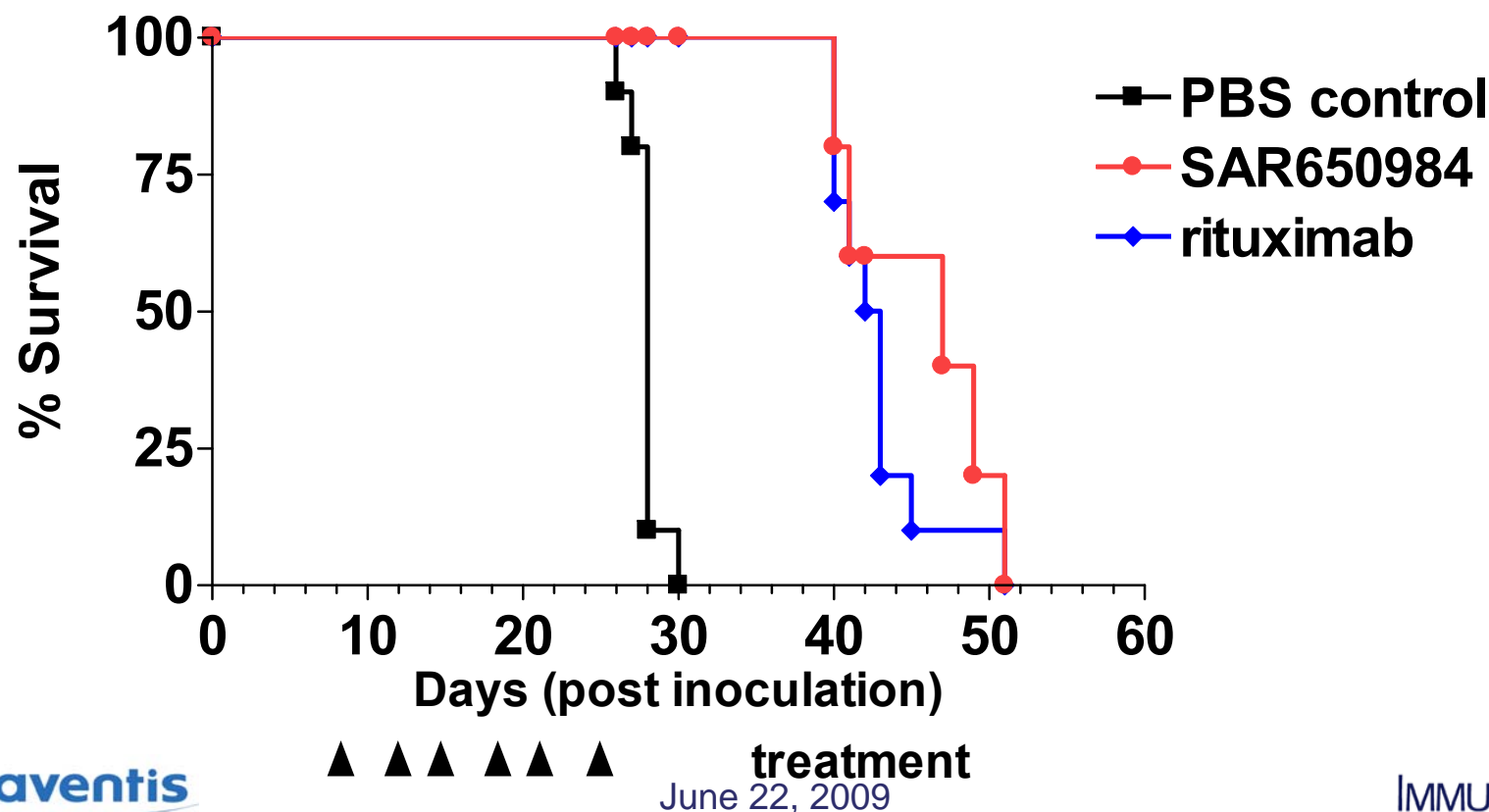
SAR650984 shows potent *in vivo* efficacy in a NCI-H929 multiple myeloma model

- Early stage NCI-H929 (CD38⁺ CD20⁻) multiple myeloma model (palpable tumors)
 - Schedule: 40 mg/kg of SAR650984 or non-binding human IgG1 control twice weekly for 3 weeks starting on day 6



SAR650984 and rituximab showed similar efficacy in a disseminated survival model with Daudi cells

- SCID mouse disseminated survival model with Daudi lymphoma cells
 - Schedule: 40 mg/kg of SAR650984 or rituximab twice weekly for 3 weeks starting on day 7
 - The median survival (range) was 28 days (26-30 days) for PBS, 47 days (40-51 days) for SAR650984, and 42.5 days (40-51 days) for rituximab.



Conclusions

- **CD38 is expressed in various hematologic disease indications**
- **SAR650984 is a humanized antibody which binds with high affinity to human CD38**
- **SAR650984 mediated pro-apoptotic, CDC and ADCC activities against a variety of cells lines derived from lymphoma, multiple myeloma and leukemia**
- **SAR650984 did not require cross-linking to induce apoptosis**
- **Cells lines with high CD38 expression were most sensitive to complement-dependent cytotoxicity by SAR650984**
- **All CD38-positive cell lines were subject to SAR650984-mediated ADCC activity regardless of antigen expression level**
- **SAR650984 showed similar in vitro CDC and ADCC activities against Raji and Daudi lymphoma cells, but displayed a higher pro-apoptotic activity than rituximab**
- **The potent *in vitro* activity translated into potent *in vivo* efficacy**
- **SAR650984 is a promising therapeutic antibody candidate for a number of hematologic malignancies**

Acknowledgements

- **ImmunoGen, Inc.: Jutta Deckert**
- **sanofi-aventis: Pascale Lejeune, Véronique Blanc**
- **Park P.U., Blanc V., Deckert, J., Lejeune P., Bartle L.M., Skaletskaya A, Mayo M.F., Zhang C., Wetzel M.-C., Tavares D.J., Lutz R.J., Chittenden T., Bissery M.-C., Lambert J.M., and Goldmacher V.S. (2008) SAR650984: A Potent Anti-CD38 Therapeutic Antibody with Three Mechanisms of Action (Apoptosis, ADCC, CDC) for Hematological Malignancies *ASH Annual Meeting, Abstract Number: 2756.***
- **Deckert, J., Yi Y., Chicklas S., Mayo M.F., Chittenden T., Park P.U. (2009) SAR650984, a humanized anti-CD38 antibody, shows potent in vitro activity by multiple mechanisms against various hematologic tumor cell lines. *AACR Annual Meeting, Abstract Number: 2048.***
- **Lejeune P., Blanc V., Courta J., Egile C., Vrignaud P., Deckert, J., Park P.U., Bissery M. (2009) In vivo therapeutic synergy of SAR650984, a humanized anti-CD38 antibody, in combination with melphalan in a multiple myeloma xenograft.. *AACR Annual Meeting, Abstract Number: 2797.***
- **Lejeune P., Deckert, J., Mayo M.F., Whiteman K., Johnson S., Guyre C.A., Blanc V., Park P.U., Lutz R.J., Bissery M., Vrignaud P. (2009) Broad spectrum of antitumor activity of SAR650984, a humanized anti-CD38 antibody targeting hematological malignancies. *AACR Annual Meeting, Abstract Number: 859.***
- **Guyre C.A., Deckert, J., Johnson S., Chicklas S., Gabriel R., Lutz R.J., Park P.U. (2009) SAR650984, a humanized IgG1 antibody targeting CD38, kills CD38-positive tumor cells through multiple mechanisms of action *in vitro* and *in vivo*. *Keystone Symposia:Antibodies as Drugs, Abstract Number: 120.***