Novel anti-myeloma immunotherapies targeting the SLAM family of receptors

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ABSTRACT
Treatment for multiple myeloma (MM) has significantly advanced in the last decade with the introduction of proteasome inhibitors and immunomodulatory therapies. Unfortunately, MM continues to cause significant morbidity and most patients eventually succumb to the disease. As in other areas of cancer, immunotherapy in MM has also evolved and holds promise to deliver long-lasting remissions or even cure. The signaling lymphocyte activation molecules (SLAM) family of surface proteins represents a group of potential targets for immunotherapy in MM as some of the family members are expressed consistently on plasma cells and also on myeloma propagating pre-plasma cells. Here, we review the SLAM family members in detail, describe their tissue distribution, biologic pathways, as well as relevant pre-clinical studies and clinical trials in MM. Our review demonstrates the value of SLAM family receptors as potential targets for anti-myeloma immunotherapies and outlines how immunotherapeutic approaches can be developed.

Introduction
Clonal plasma cell dyscrasias represent a continuum of disorders from monoclonal gammopathy of unknown significance (MGUS) through smoldering myeloma (SMM) to multiple myeloma (MM) and plasma cell leukemia. In the case of MM, the last decade has witnessed unprecedented improvements in overall survival rates. However, patients still undergo multiple lines of treatment and most will eventually suffer a fatal relapse. In particular, the treatment of MM patients with high-risk cytogenetics has been disappointing and so far we have not been able to identify a convincing way of dealing with this more therapy-resistant and aggressive subtype of myeloma. Immunotherapies targeting certain surface antigens carry the potential to overcome high relapse rates and the more aggressive clinical course of high-risk MM because these novel approaches potentially target the tumor cell irrespective of its biologic characteristics.

One major prerequisite for the design of novel immunotherapeutic approaches is the identification of promising target antigens. We have defined several criteria, which we deem essential for defining the most promising myeloma targets. We believe that an ideal myeloma-associated antigen for antibody-based approaches
1) must be expressed on the surface of myeloma cells
2) should be expressed by as few normal tissues as possible
3) should be expressed homogeneously on tumor cells by a sufficiently large proportion of myeloma patients.
4) should have a central function in the biology and/or pathophysiology of myeloma to prevent its downregulation under the selection pressure of an effective immunotherapy

Signaling lymphocyte activation molecule (SLAM) family receptors are expressed on the surface of different immune cells, they function as activating and inhibitory signal transducers, and they can potentially be used as targets for novel immunotherapies for MM and other lymphoid malignancies. In this review, we will determine whether the individual members of the SLAM family of receptors fulfill the criteria listed above, we will summarize the relevant pre-clinical data and the most recent clinical trials using SLAM family members as targets, and we will demonstrate how SLAM-targeting approaches can be designed. Overall, our summary will help to answer the question if and how members of the SLAM family of receptors can become part of the growing armamentarium in the fight against MM.

The signaling lymphocyte activation molecule (SLAM) family of receptors
Most SLAM family receptors including CD84, CD150, CD229, CD244, CD319 and CD352 are type 1 transmembrane receptors, while only SLAM family member CD48 has a glycosyl phosphatidyl inositol (GPI) membrane anchor. All SLAM family receptors show a broad expression on different immune cells (Table 1), and they share a similar structure with an extracellular domain consisting of an Ig variable (V)-like domain, an Ig constant 2 (C2)-like domain, a transmembrane domain, and a cytoplasmic tail. SLAM family member CD229 represents somewhat of an exception because it has 4 Ig-like domains with two tandem repeats of the basic V-like and C2-like organi-
The SLAM family and multiple myeloma

The SLAM family of receptors has been implicated in the pathogenesis of different diseases including chronic infections, autoimmune diseases and cancer. The SLAM family has a total of nine members; however, studies in MM have been limited to CD150 (SLAMF1), CD48 (SLAMF2), CD229 (SLAMF3), CD244 (SLAMF4), CD352 (SLAMF6) and CS1 (SLAMF7). We will review these SLAM family members in the context of MM including an overview of their tissue distribution, postulated mechanisms of action, and clinical studies that have been reported or are ongoing.

CD48 (SLAMF2) and CD244 (SLAMF4, 2B4)

CD48 is a GPI-linked glycoprotein expressed on the surface of immune cells and acts as the ligand for CD244. It does not have a cytoplasmic tail with ITSMs like other SLAM family proteins but modulates NK cell functions together with CD244. CD48 is highly expressed on T cells, B cells and plasma cells, and at a lower level on monocytes, neutrophils and CD34+ cells, but it is absent from platelets and red blood cells (Table 1). Hosen et al. have shown that CD48 is expressed on more than 90% of plasma cells in 22 out of 24 MM patients with MM they studied. In vitro analyses using murine anti-human CD48 antibody showed complement-mediated cytotoxicity against myeloma cells, T cells and B cells, while CD34+ stem cells were spared. A significant anti-myeloma effect was also seen in a xenograft MM model.

CD48 on NK cells can act both as a stimulatory and inhibitory signal; however, the exact pathways underlying this are not well understood. Different groups have demonstrated contradictory findings whether CD244 engagement with CD48 on target cells decreases or enhances NK cell cytotoxicity. Recent studies on the role of CD244 on T cells show that it might act as an inhibitory signal. In chronic infections, like Hepatitis B and tuberculosis, T cells show an increased expression of CD48 and decreased CD8+ T-cell cytotoxic activity. In contrast, blocking of CD244 or CD48 resulted in an enhanced CD8+ T-cell cytotoxicity. Fauriat et al. showed that CD244 is downregulated on NK cells of patients with MM. They analyzed six patients with MM and found that CD244 expression by flow cytometry was significantly lower compared with healthy donors and postulated that this could be one mechanism of immune escape by MM. Costello et al. reported a decrease in the bone marrow NK cell expression of CD244 and NKG2D and NKP30 compared with the peripheral blood in patients with MGUS. Both of these studies suggest that there is an immune escape mechanism with downregulation of NK cell activating receptors in the bone marrow environment in MM.

Overall, we think that CD244 and CD48 are interesting targets for MM, even though their exact role in the pathogenesis of MM is not understood. Unfortunately, CD48 is expressed on myeloid lineage and CD34+ stem cells, albeit at a lower level compared with MM, and this could lead to undesired myelosuppression if CD48 is directly targeted using novel cytotoxic immunotherapies.

CD229 (SLAMF3, Ly9)

CD229 is expressed on the surface of normal T and B lymphocytes (Table 1) as well as on NK cells. CD229 is unique among members of the SLAM family as it has four extracellular Ig domains and the longest cytoplasmic tail containing two ITSMs. Signaling is mediated by SAP binding to the two ITSMs.

Table 1. Tissue distribution of SLAM proteins and their ligands.

| SLAM # | Name | Expression | Ligands | Potential target? |
|-------|------|------------|---------|------------------|
| SLAMF1 | CD150 | T, B, Mo, Mφ, DC, PC | Self | |
| SLAMF2 | CD48 | Pan-leukocytes, endothelial cells | CD244 | |
| SLAMF3 | CD229/Ly9 | T, B, HSC, Mo, Mφ, NKT, malignant PC | Self | |
| SLAMF4 | CD244/2B4 | NK, NKT, T, DC, eosinophils, Mo, mast cells | CD48 | |
| SLAMF5 | CD84 | B, T, Mo, platelets, mast cells, eosinophils | Self | |
| SLAMF6 | CD352/NTB-A | B, T, NK, NKT, Mφ, DC | Self | |
| SLAMF7 | CD319/CS1/CRACC | B, T, Mo, NK, DC, NKT, malignant PC | ND | |
| SLAMF8 | CD353/BLAME/ND | B, T, DC, Mφ, neutrophils, eosinophils, Mo, DC | ND | |
| SLAMF9 | CD2F-10 | Self | ND | |

B: B cells; DC: dendritic Cells; HSC: hematopoietic stem cells; Mo: monocytes; Mφ: macrophages; ND: not determined; NK: natural killer cells; NKT: natural killer T cells; PC: plasma cells; T: T cells. Color coding for potential targets: green: promising target; yellow: inconclusive evidence; and red: not promising target.
on the cytoplasmic tail and CD229 homophilic binding leads to interaction of SAP and EAT-2 similar to other SLAM family receptors. Knockdown of CD229 in mice results in normal development of T cells, B cells, NK cells and natural killer T (NKT) cells but mice show a mild defect in T-cell activation and induction of a Th2-type response. In addition, CD229 is the only SLAM member that is endocytosed and recycled to the cell surface mediated by adaptor protein (AP)-2 and clathrin coated pits. This internalization is mediated by interaction of tyrosine residues in the cytoplasmic tail of CD229 with Grb2. It has also been shown that CD229 activation can downregulate TCR signaling and that Grb2 expression by TCR signaling leads to internalization of CD229, thereby allowing TCR activation. Finally, there is evidence that CD229 modulates the development of an innate-like T and B cells response.

Our group has studied the expression of CD229 in 27 patients with plasma cell dyscrasias, which included 49 patients with MM, 7 with SMM, 17 with MGUS and 4 with plasma cell leukemia. The tumor cells of all patients including newly diagnosed and relapsed patients showed strong expression of CD229. Furthermore, we observed that even though CD229 is known to be expressed on other hematopoietic cells like NK, T and B cells, its expression was weaker on these normal lymphocytes compared with myeloma cells. In addition, CD229 was highly expressed in patients with plasma cells with aberrant expression of CD56. Finally, we also showed that CD229 is expressed on the CD19 “CD138+” population of myeloma cells, which can be regarded as the myeloma-propagating pre-plasma cells that contributes to relapse and refractory disease.

High CD229 expression on myeloma was also reported by Yamada et al. in a study of 144 newly diagnosed and 25 relapsed/refractory MM patients. In myeloma cell lines they also showed that CD229high cells had a higher proliferation rate compared with CD229low cells. In addition, they demonstrated that anti-myeloma chemotherapy melphalan was less capable of inducing apoptosis in CD229high cells compared with CD229low cells. Finally, they confirmed that CD229 was expressed on the CD138-immature myeloma cell population similarly to our own study. Carulli et al. studied the expression of CD229 in 40 patients with MM, 8 at the time of diagnosis, 8 at stringent complete response and the other 24 at partial response (PR) or VGPR. Malignant plasma cells from all these patients showed CD229 expression irrespective of treatment or response.

Our group was the first to show that targeting CD229 using antibodies can induce significant NK cell-mediated ADCC as well as CDC in in vitro models of MM. Furthermore, knockdown of CD229 in myeloma cell lines led to increased apoptosis and decreased colony formation, implying that CD229 is essential for the growth of MM cells. As of now there are no clinical studies with CD229-targeting antibodies or T-cell therapies, but the preclinical results available augur such novel immuno-therapeutic approaches in the near future.

**CD352 (SLAMF6, NTB-A)**

CD352 is expressed on normal NK cells, T cells and B lymphocytes and signals through the ITSM-SAP pathway. CD352 signaling promotes Th1 responses by T cells and leads to proliferation and IFN-γ production, and CD352 receptors also enhance NK cell cytototoxicity. Lewis et al. studied the expression of CD352 in myeloma and showed that 13 of 15 patients evidenced surface expression by flow cytometry. Lewis et al. recently reported their preclinical study of a new CD352-targeting antibody drug conjugate SGN-CD352A, a humanized anti-CD352 antibody conjugated with two molecules of the DNA-damaging agent pyrrolobenzodiazepine dimer. They found that SGN-CD352A displayed potent cytotoxicity against human myeloma cell lines with minimal toxicity to normal human T cells and B cells. Similar efficacy was demonstrated in mouse xenograft models of MM with 90–100% complete responses using the antibody conjugate. We consider CD352, an interesting target with an expression limited to the lymphoid compartment and high expression in MM, although it is less well studied in MM compared with SLAMF7 and SLAMF3.

**CD319 (SLAMF7, CS1, CRACC)**

CS1 exists as two isoforms CS1-long (CS1-L) and CS1-short (CS1-S) both of which are expressed (Table 1) on the surface of different normal immune cells. CS1 is unique among the SLAM family in that it does not bind to SAP but expression is mediated through the EAT-2 pathway. CS1-S lacks the EAT-2 binding tyrosine residues and it is not clear how CS1 regulates cellular function. Knockdown of CS1 in NK cells leads to a decrease of their cytotoxic function toward both CS1-positive and CS1-negative target cells. Most of the studies show that surface expression of CS1 among normal tissues is restricted to immune cells like NK, B and T cells. However, tissue-specific mRNA expression also shows expression of CS1 in the kidney, pituitary, heart, skeletal muscles and parts of the brain. CS1 expression on B cells varies, and it is higher during the later stages of development including mature plasma cells. EAT-2 is absent from T cells, B cells and plasma cells and so the mechanism of CS1 signaling in these cells is not well understood. Tai et al. reported expression of CS1 on most MM cell lines and also observed its expression on plasma cells of patients with MM including patients with relapsed/refractory disease and adverse cytogenetic abnormalities.

Importantly, early preclinical results had indicated that serum levels of soluble CS1 correlate with the stage and activity of MM and that an anti-CS1 antibody (HuLuc90) inhibits adhesion of plasma cells to bone marrow stromal cells and contributes to anti-MM activity. Furthermore, antibody-dependent cell-mediated cytotoxicity (ADCC) could be induced both in vitro and in vivo using anti-CS1 antibody, and cytotoxicity was augmented when the effectors were pre-treated with lenalidomide. In parallel, Hsi et al. reported similar findings with uniform CS1 expression on myeloma cells and the anti-CS1 antibody HuLuc63 inducing NK-cell-mediated ADCC in vitro and in vivo. In addition to the direct ADCC by binding to myeloma cells, anti-CS1 antibody can bind to CS1 on NK cells and activate these effector cells, thereby enhancing ADCC to myeloma but not to autologous NK cells. This combined pre-clinical work set the stage for clinical studies with anti-CS1 antibodies.

Elotuzumab (previously called HuLuc63) is an IgG1 humanized antibody against CS1, which has been evaluated in clinical
trials. Despite the promising pre-clinical data, single agent elotuzumab did not result in any significant clinical benefit. In the first phase 1 multicenter dose escalation study, Zonder et al. treated 35 patients with relapsed/refractory MM: no objective response was seen and 9 patients had stable disease but MM progressed in the others.38

Based on pre-clinical studies showing superior anti-MM activity when elotuzumab was combined with novel agents such as bortezomib, several combinatorial clinical trials were initiated.39 Accordingly, Jakubowiak et al. studied the combination of elotuzumab with bortezomib in a dose-escalation phase 1 study in patients with relapsed/refractory MM. Of the 27 evaluable patients treated, a partial response or better was seen in 48% with a median progression-free survival (PFS) of 9.5 mo.40 The same group later studied the effect of combining elotuzumab with bortezomib and dexamethasone in an open label randomized phase 2 study of 152 patients with relapsed/refractory MM. They treated 40 heavily pretreated patients with previously received lenalidomide.4 This led to further trials. Despite the promising pre-clinical data, single agent elotuzumab did not result in any significant clinical benefit. In the first phase 1 multicenter dose escalation study, Zonder et al. treated 35 patients with relapsed/refractory MM: no objective response was seen and 9 patients had stable disease but MM progressed in the others.38

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Elotuzumab has also been studied in combination with thalidomide and dexamethasone. Mateos et al. recently reported results of a phase 2 single arm study where patients were treated with elotuzumab, thalidomide and dexamethasone with the provision of adding cyclophosphamide in case of an insufficient response. They treated 40 heavily pretreated patients on this protocol and observed an overall response rate (ORR) of 38% with a median time to response of 1.9 mo. Interestingly a 32% ORR was observed in patients previously refractory to IMIDs. They observed a median PFS of 3.9 mo and a median survival of 16.3 mo, which for this patient population is very encouraging.43

The role of elotuzumab in upfront treatment of newly diagnosed patients with MM is currently being studied. The ELOQUENT-1 study (NCT01891643) is an ongoing Phase III trial with lenalidomide, dexamethasone with and without elotuzumab in newly diagnosed MM patients who are not candidates for high-dose therapy and autologous stem cell transplantation due to either comorbidities or advanced age. Two studies have been opened with a CS1-specific antibody–drug conjugate (ABBV-838), one in combination with venetoclax and dexamethasone (NCT02951117) and the other as a dose-escalation study with ABBV-838 monotherapy in relapsed refractory MM (NCT02462525).

Antigen CS1 has also been used as a target for cellular anti-myeloma immunotherapies. For example, chimeric antigen receptors (CARs) targeting CS1 have been incorporated into T cells and NK cells (Fig. 1). This approach has resulted in remarkable efficacy in vitro and in a xenograft model of MM.

![Figure 1. Different types of SLAM-specific immunotherapies. The figure shows different ways to target the SLAM family of receptors expressed on myeloma cells. The myeloma antigen can be targeted by monoclonal antibodies (mAb), antibody-drug conjugates (ADC), bispecific antibodies, specific T cells recognizing HLA/SLAM peptide complexes, and SLAM-specific chimeric antigen receptor (CAR) T cells.](image-url)
only be targeted by antibody-based approaches but that a novel
strategy toward myeloma cell lines and also primary MM cells.47
Chu et al. retrovirally transduced T cells and NK cells with anti-
CS1 CARs and were able to show significant survival advantage
in a mice treated with these CS1-specific CAR T cells and NK
cells compared with mock transduced T and NK cells. Even
though CS1 is expressed on T cells and NK cells themselves,
the CAR T cells did not have a significant cytotoxicity toward
these normal lymphocytes, the mechanism of which is not
completely understood.44,45 On the contrary, Danhof et al.
showed that there was toxicity to CS1-expressing T and NK
cells upon co-culture with CS1-specific CAR T cells. They fur-
ther went on to show that it was possible to generate potent
cytotoxic CS1-specific CAR T cells from autologous T cells of
MM patients.46 Moreover, Bae et al. reported that CS-1 cannot
only be targeted by antibody-based approaches but that a novel
HLA-A2-restricted CS1 peptide induced CS1-specific CTLs
against MM. These CTLs displayed significant cytotoxicity
toward myeloma cell lines and also primary MM cells.47
Overall, we consider CS1 a reasonable target for anti-mye-
loma immunotherapies as it is expressed in most patients with
MM including relapsed/refractory. However, it is unknown
whether the gene expression of CS1 in non-immune cells can
lead to low-level surface expression not detected by available
immunophenotyping techniques and toxicity especially with
more potent cellular immunotherapies like CAR T cells and
CTLs. The significant RNA expression of CS1 in different epi-
thelial tissues also represents a potential toxicity issue. Further-
more, it is not known whether CS1 plays an essential role in the
pathogenesis of MM and, therefore, it is possible that its down-
regulation under the selection pressure of an effective immuno-
therapy will lead to immune escape.

CD150 (SLAMF1), CD84 (SLAMF5), CD353 (SLAMF8, BLAME), CD2F10 (SLAMF9)

Studies on SLAM family receptors CD150, CD84, CD353 and
CD2F10 in MM are limited and they do not seem to be inter-
esting targets in MM. The available data for these four recep-
tors are reviewed below.

CD150 is expressed on T cells, B cells, NK cells and dendritic
cells.48 In addition, CD150 seems to be expressed on normal
and abnormal plasma cells (Table 1). Schoenhals et al. showed
that CD150 was uniformly expressed on malignant plasma cells
in all 10 MM patients studied.49 However, Gordienko et al. did
not detect any CD150 expression in MM cell line RPMI-
8226.50 Thus, it remains an open question if and why CD150 is
only expressed on a subset of myeloma cells. No thera-
peutic interventions have been pursued and no studies are
available, considering CD150 a target for cancer immuno-
therapy.

CD84 is expressed on a wide range of hematopoietic cells
including T cells, B cells, dendritic cells, monocytes, macro-
phages, neutrophils, eosinophils, mast cells, basophils, platelets
and hematopoietic stem cells.51,52 CD84 is differentially
expressed on B cells and may play a role in the activation of
memory B cells.53 It has also been shown that CD84 plays an
important role in the interaction between T cells and B cells by
prolonging cell contact, enhancing T follicular helper cell func-
tion, as well as the formation of germinal centers.54 Studies
have been contradictory with regards to the expression on
plasma cells. Tangye et al. showed a loss of expression of CD84
when plasmablasts were generated in vitro similar to their find-
ing of absent CD84 expression on plasma cells and myeloma
cell lines.53 In contrast, De Salort et al. showed that CD84 was
present in up to 70% of plasma cells residing in splenic or ton-
sillar tissue.[55 There are no studies reporting the presence of
CD84 on malignant plasma cells in MM. As CD84 is expressed
on most hematopoietic cells including CD34+ stem cells, we do
not think that it would be a preferable target for immune-based
therapies.

CD353 is a type 1 transmembrane protein with an extracel-
lar and transmembrane domain but unlike most other SLAM
members has a short cytoplasmic tail that does not contain
ITSMs that mediate SAP dependent activity.56 CD353 is
expressed on monocytes, dendritic cells and neutrophils and
plays an important role as a negative regulator of reactive oxy-
gen species (ROS) production and cell migration during
inflammation.57 Llinas et al. found that CD353 was only faintly
expressed on B cells by flow cytometry and not expressed on T
cells and NK cells. Expression on plasmablasts and plasma cells
was also limited, with only 14% of cells expressing low level of
CD353.58 There are no studies that have specifically looked
into the expression of CD353 on myeloma plasma cells and
with its predominant expression on myeloid lineage we do not
think that CD353 would be an interesting target for MM immuno-
therapy.

CD2F10 is the least studied of all the SLAM proteins and its
function is not understood. Similar to CD353 its short cyto-
plasmic tail does not have ITSMs. Its tissue distribution as
determined by RT-PCR is predominantly on immune cells like
monocytes, dendritic cells, T and B cells but the role it plays in
the biology of these cells is not understood.59 There are no
studies looking at the surface expression of CD2F10 on plasma
cells in MM and so we do not consider CD2F10 a promising
target for the immunotherapy in MM.

Conclusions and future directions

The SLAM family of receptors has nine members, which are
mostly expressed on immune cells. To be efficient targets for
immunotherapy the target antigens should have limited expres-
sion on normal immune cells and should be consistently
expressed on the surface of malignant plasma cells. Of the nine
SLAM proteins only certain members like CS1, CD48, CD352
and CD229 are highly expressed on plasma cells of patients
with MM. The uniform and strong expression in all stages of
MM, even the relapse refractory disease, makes these SLAM
family proteins suitable targets for different immunotherapies
such as monoclonal antibodies, bispecific antibodies, immuno-
conjugates, cytotoxic T cells, and CAR T cells (Fig. 1). The idea
for SLAM-directed therapies to target the chemotherapy-resis-
tant myeloma cell subpopulation in addition to the bulk of MM
cells seems particularly attractive and needs to be explored in
clinical trials. One could imagine, for example, that high-dose
chemotherapy followed by autologous stem cell transplant
could be used to perform tumor debulking followed by an anti-
SLAM immunotherapy that will eradicate the remaining che-
motherapy-resistant minimal residual disease.
The only caveat with SLAMF receptors as targets for immunotherapies is that the SLAM members are not exclusively expressed on MM but also on other hematopoietic cells albeit at a lower level. While it remains to be seen whether expression on healthy cell types would result in clinically relevant toxicities, several strategies to limit off-tissue reactivity have been developed. In the case of CAR T cells, for example, the incorporation of inducible suicide genes would allow the rapid deletion of engineered T cells as soon as toxicities are observed. In addition, different combinatorial strategies have been developed to improve the tissue specificity of receptor-transgenic T cells, which require either the binding of the CAR to multiple simultaneously expressed antigens or the absence of antigens binding to inhibitory CARs.

Based on their efficacy and potential long-term persistence we believe that engineered CAR T cells represent the preferable approach out of the different SLAM-targeting antibody-based approaches (Fig. 1). Unfortunately, downregulation of the target antigen with subsequent antigen escape has become a clinical issue. We believe that this problem can be overcome if we focus on targets, which play a central role in the biology of the tumor of interest. In the case SLAM family members, CD229 is probably the only candidate where available data point to an anti-apoptotic role in MM. In our view CD229 should, therefore, be among those SLAM family members preferably being investigated as targets for anti-myeloma immunotherapies.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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