CD19-based immunotherapy for B cell malignancies and Autoimmune diseases

Hajar Abbasi
MSc student of immunology

Supervisor: Dr. Tohid Kazemi

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Introduction

- B lymphocytes are the origin of humoral immunity, contribute to autoimmunity, and represent a substantial proportion of hematopoietic malignancies.

- Consequently, cell surface molecules expressed by B cells and their malignant counterparts represent important targets for immunotherapy.

- B-lymphocyte-directed immunotherapies, such as anti-CD20 antibody rituximab, have demonstrated activity in both B-cell malignancies and autoimmune diseases.
• CD20 immunotherapy depletes mature B cells but does not effectively deplete pre-B or immature B cells, some B cell subpopulations, antibody-producing cells, or their malignant counterparts.

• Despite the effectiveness of this therapy, novel treatment approaches are still highly needed.
CD19 is emerging as a promising target due to:

1. Has a broader expression profile than that of CD20.
2. Is expressed on pre-B cells while CD20 is not.
3. Its expression persists longer on B cells during their maturation.
4. Unlike CD20, CD19 is rapidly internalized.
Properties of CD19:

- Type I transmembrane glycoprotein belonging to Ig superfamily
- Molecular weight: 95 kD
- Length of protein: 556 aa
- Gene location: 16p11.2
- Length of gene: 7.41 kb
- Is expressed under the control of BSAP
CHr 16

**p11.2**

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**SP**: signal peptide, **Ig**: Ig-like domain, **TM**: transmembrane domain
CD19 molecular structure:

**Figure 1 CD19 molecular structure.** CD19 is a type I one-pass transmembrane protein. The two extracellular C2 Ig-like domains are separated by a small helical non-Ig domain with possible disulfide links. The highly conserved, 242 amino acid cytoplasmic domain includes multiple tyrosine residues. Three key tyrosine residues are shown with their associated signaling kinases and molecules.
Expression of CD19:

- CD19 is only expressed by B cells and FDC, not T cells or NK cells, monocytes/macrophages, neutrophils, RBCs, or platelets.

- CD19 is expressed by early pre-B cells from the time of heavy chain rearrangement until terminal differentiation into plasma cells.

- Pax5 and CD81 are required for the normal expression of CD19.
Function of CD19

- CD19 functions as a co-receptor of BCR in interaction with CD21, CD81 and CD225.

- As a component of the B-cell receptor complex, CD19 regulates the threshold for B-cell activation.
Figure 2 CD19 associated signaling complex. Antigen-C3d complexes can engage the CD19/21 complex in both a BCR-independent or BCR-dependent fashion. The CD19 complex includes complement receptor CD21, which binds C3d-modified antigen.
• The importance of CD19 in the development and function of B cells was demonstrated through gene targeted or transgenic mice.

- **CD19-deficient (CD19^−/−) mice:**
  - B cells are hyporesponsive to transmembrane signals
  - B cells generate modest immune responses
  - Fail to develop germinal centers
  - Decreased number of B1 cells
  - Have < 10% of the normal levels of serum IgG.

- **Over-expression of CD19 in transgenic mice results in:**
  - B cell that are hyperresponsive to transmembrane signals
  - Elevated humoral immune responses
  - Diminished number of B cells in peripheral pool
  - Increased frequency of B1 cells within the peritoneum and spleen.
CD19 expression by B cell malignancies & autoimmune diseases:

- Evaluation of anti-CD19 therapy as a therapeutic option requires careful consideration of CD19 expression levels by B cells from patients.

- Patients with systemic sclerosis displayed a ~20% increase of CD19 expression on peripheral blood B cells.

- Dysregulated CD19 expression has been reported in patients with systemic sclerosis, SLE and ANCA-associated vasculitis.

- The majority of B cell malignancies express CD19 at normal to high levels (pre-B ALL, common-ALL, null-ALL, CLL, hairy cell leukemias and non-Hodgkin’s lymphomas.)
Types of CD19-targeted agents:

- Bispecific antibodies
- Antibody-Drug conjugates
- Fc-engineered antibodies
- CD19-chimeric antigen receptors
Bispecific Antibodies
**Blinatumomab:**

- **Sponsor:** Amgen
- **Format:** BiTE
- **Mechanism of action:** T-cell recruitment and activation
- **Indication:** ALL, DLBCL
- **Phase:** 2

- **B lineage-specific anti-tumor mouse monoclonal antibody.**

- **Blinatumomab** is a 55 kDa fusion protein composed of two scFv.

- It is designed to recruit CD3 cytotoxic T cells to lyse CD19-expressing B cells.
The transient close association of the B cell with the T cell

- occurs in an antigen independent manner
- results in T cell mediated lysis through the production of perforin and granzyme B
- also results in a CD19 dependent polyclonal expansion of T cells and release of cytokines (TNF-α, INF-γ, and IL-2)

In clinical studies, blinatumomab has been evaluated in NHL, ALL, ALL (MRD+) and DLBCL.
AFM11:

- Sponsor: Affimed
- Format: RECUIT TandAb
- Mechanism of action: T-cell recruitment
- Indication: NHL
- Phase: preclinical

Advantages of TandAb:

- lack of Fc-mediated side effects.
- Specific engagement of NK-cells and T-cells.
- With a molecular weight of approximately 110 kDa, TandAbs are far above the first-pass renal clearance limit.
• AFM11 binds to CD3 and CD19 and is comprised of fully human binding domains, which are isolated as scFvs from a phage-display library.

• This molecule is currently in the preclinical development stage for treatment of NHL.
AFM12:

- Sponsor: Affimed
- Format: RECUIT TandAb
- Mechanism of action: NK-cell recruitment
- Indication: NHL
- Phase: preclinical

AFM12, is a bispecific, tetravalent human antibody specifically designed to treat Non-Hodgkin Lymphoma

AFM12 specifically constructed to target CD19 as a tumor target and CD16A on NK-cells as immune effector cells.
CD19xCD3 DART

- Sponsor: Macrogenics
- Format: DART
- Mechanism of action: T cell recruitment
- Indication: B cell lymphoma
- Phase: preclinical

- DARTs consist of two distinct polypeptides that are coexpressed to generate a covalently-linked heterodimeric complex with one binding site for each of two specificities.

- Advantages of DART format:
  - The DART format provides additional stabilization through a C-terminal disulfide bridge.
  - This format lacks an intervening linker sequence and thus more analogous to the natural association in an IgG molecule.
Antibody-Drug conjugates
What makes an optimal ADC?

Antibody
• Target recognition unaltered compared with naked Ab
• Abundant target expression and internalization

Drug
• Highly potent antitumor agent (sub-nM IC90 as a free drug)
• Validated mechanism of action (microtubule inhibition or DNA damaging)

Linker
• Stable in plasma to avoid premature release of the drug
• Labile once internalized to release the drug in its active form
SAR3419

- Sponsor: Sanofi-Aventis
- Format: Antibody-drug conjugate (ADC)
- Mechanism of action: Delivery of toxic payload
- Indication: DLBCL, ALL, NHL
- Phase: 2

SAR3419 is an ADC that consists of a humanized monoclonal IgG1 antibody (huB4) attached to a highly potent tubulin inhibitor, the maytansinoid DM4.
• Maytansine and other maytansinoids are antimitotic agents that bind to tubulin, inhibiting microtubule assembly and inducing G2/M arrest in the cell cycle, which subsequently leads to cell death.

• SAR3419 contains an average of 3.5 DM4 molecules per molecule of antibody.
Combotox

- Sponsor: Montefiore Medical Center
- Format: scFv immunotoxins
- Mechanism of action: Delivery of toxic payload
- Indication: ALL
- phase: 1

- Combotox is a mixture of two deglycosylated ricin-A -conjugated monoclonal Abs (RFB4 and HD37) directed against CD19 and CD22, respectively.

- RTA’s native form is N-glycosylated and hence mannose and fructose receptors on liver cells can recognize and bind the RTA protein of iTs, thus shortening their half lives and causing liver damage. For this reason, RTA has been chemically deglycosylated (dgRTA) before its conjugation.

- The mechanism of action of ricin in the body is inhibition of protein synthesis.
DT2219ARL

- Sponsor: NCI
- Format: Bispecific immunotoxin
- Mechanism of action: Delivery of toxic payload
- Indication: B cell malignancies
- phase: 1

- DT2219ARL, a bispecific immunotoxin targeted to CD19 and CD22, is composed of two scFv antibodies and a truncated form of a diphtheria toxin (DT390).

- DT390 contains the A fragment of native DT that catalyzes ADP ribosylation of EF-2 leading to irreversible inhibition of protein synthesis and cell death.
SGN-CD19A

- Sponsor: Seattle Genetics
- Format: ADC
- Mechanism of action: Delivery of toxic payload
- Indication: NHL, ALL
- phase: 1

- SGN-CD19A is composed of a humanized antibody conjugated to Monomethyl Auristatin E (MMAE) via a protease-sensitive peptide-based linker.

- Monomethyl Auristatin E is an antimitotic agent which inhibits cell division by blocking the polymerisation of tubulin.
Fc-engineered Antibodies
MEDI-551

- Sponsor: Medimmune
- Format: Glyco-engineered antibody
- Mechanism of action: Enhanced ADCC
- Indication: DLBCL, CLL, MS
- phase: 2

- MEDI-551 is an Fc-engineered humanized CD19 antibody with enhanced ADCC.

- Because the antibody is produced in a fucosyltransferase-deficient cell line, it is afucosylated and therefore has increased binding to FcγRIIIa.

- One of the most successful modifications to the Fc carbohydrate structure has been the removal of fucose, which results in an improved binding interaction with the FcRIIIa carbohydrate.
MDX-1342

- Sponsor: Bristol-Myers Squibb
- Format: Glyco-engineered antibody
- Mechanism of action: Enhanced ADCC
- Indication: CLL, RA
- phase: 1

- MDX-1342 is an Fc-engineered human anti-CD19 antibody with enhanced ADCC.

- Similarly to MEDI-551, it is an afucosylated antibody produced in a fucosyltransferase-deficient cell line.
XmAb-5574/MOR-208

- Sponsor: Morphosys/Xencor
- Format: Fc engineered antibody
- Mechanism of action: Enhanced ADCC
- Indication: CLL
- phase: 1

- XmAb5574 is a novel engineered anti-CD19 mAb with a modified Fc domain designed to enhance binding of FcγRIIIa which results in improved ADCC activity.

- The ADCC is mediated by NK cells through a granzyme B-dependent mechanism.
• In contrast to MEDI-551 and MDX-1342, XmAb-5574’s Fc modification involves changes in the protein sequence of the Fc domain rather than the glycosylation pattern.

• The modified Fc domain of XmAb-5574 with 2 amino acid substitutions S239D and I332E enhances cytotoxic potency of Ab by increased affinity for FCγRIIIa and diminished binding to FCγRIIb.
XmAb-5871

- Sponsor: Xencor/Amgen
- Format: Fc engineered antibody
- Mechanism of action: B cell inhibition via CD32B
- Indication: RA, SLE
- phase: 1

- XmAb-5871 is a humanized, Fc-engineered, anti-CD19 antibody for the treatment of autoimmune diseases.

- The antibody’s Fc domain selectively binds FcγRIIB (CD32B), an inhibitory receptor on B cells which leads to suppression of B cell activity upon coengagement.

- This Ab stimulated phosphorylation of the ITIM of FcγRIIB.
Anti-CD19-CAR

• CARs are artificial T-cell receptors constituted by an Ag-recognizing extracellular domain derived an Ab molecule linked to a T-cell triggering domain.

• CARs are generated by joining the VL and VH regions of a monoclonal antibody, expressed as a scFv molecule, to an intracellular signaling domain, usually the zeta-chain of the TCR/CD3 complex or the γ-chain from the FcεRI receptor.

• Typical production of CARs involves collecting peripheral T cells from a patient, genetically engineering the cells to express the chimeric receptor and expansion ex vivo.
Advantages of CAR:

• CARs recognize tumor antigens in a human HLA independent manner.

• This allows CAR-modified T cells to overcome the tumor’s ability to escape immunodetection by down-regulation of HLA molecules on the cell surface.

• Furthermore, because tumor targeting is HLA-independent, the use of CARs is applicable to a broad range of patients irrespective of HLA-type.
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<th><a href="http://www.clinicaltrial.gov">www.clinicaltrial.gov</a> identifier</th>
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MDACC indicates MD Anderson Cancer Center; NIH, National Institutes of Health; FHCR, Fred Hutchinson Cancer Research Center; NHL, non-Hodgkin lymphoma; and scFv, single-chain variable fragment.

*Donor-derived EBV-CTLs.
†Donor-derived EBV CTLs.
‡Donor-derived CD8+ central memory viral specific (EBV or CMV) T cells.
Summary

- CD19 immunotherapy is emerging as a promising approach for B cell malignancies, as well as inflammatory diseases.

- The potential of CD19 as an immunotherapeutic target was recognized many years ago, but initial clinical trials with mAbs to CD19 did not result in durable effects despite demonstrating responses in some patients.

- A number of approaches have been attempted to improve the activity of CD19-directed therapy, including bispecific antibodies, ADCs, Fc-engineered antibodies and CARs.

- Blinatumomab is expected to be the first CD19 agent to reach the market in 2014 for the treatment of ALL.
Thanks for attention