Catalytic antibody aldolase 38C2: rationale and biomedical applications

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1. Introduction

Catalytic antibodies, sometimes also referred to as "abzymes", are antibodies that possess catalytic activity. The concept of catalytic antibody is based on Pauling's "transition state theory", according to which enzymes catalyze a given reaction by stabilizing the transition state. In the 1980s, Richard Lerner and colleagues showed that by using transition state analogues as immunogens, it is feasible to trigger enzymatic activity in antibodies. Later on, alternative approaches for generation of catalytic antibodies have been developed, and naturally occurring catalytic antibodies have been found in the context of many diseases, displaying both pathologic and beneficial roles.

2. Aims and methods

Starting question: "Can catalytic antibodies (and if they do, how) be considered a paradigmatic example of how closely basic research is intertwined with knowledge of pathology / design of new therapeutic strategies?"

Aim: to find an example of catalytic antibody to answer the question. Four areas of interest (Methods of generation, Reaction mechanisms, Natural occurrence in pathology and Versatility of applications) were defined to guide research and achieve an overall vision of the field.

The PubMed database was searched using the words "catalytic antibody", initially, and "aldolase 38C2", once the example of catalytic antibody had been selected. In both cases, reviews were chosen based on publication date and quality of the journal. Later, relevant original papers were found thanks to the knowledge acquired and also by using the ISI Web of Knowledge.

3. Characteristics of 38C2

- Mimics class I natural aldolases -> bears a reactive lysine
- Produced by "reactive immunization": the immunogen is actually a chemical reaction -> enamine formation between the desired lysine and a β-1,3 diketone hapten (Fig.1)
- Has broad scope specificity and K_{cat}/k_M comparable to natural aldolases
- Reactive lysine is found in framework region 3 of the variable domain of the heavy chain
- Structure of related 33F12 antibody (92% identity) -> LysH93 lies at the bottom of a 11 Å deep cleft (Fig.2)
  - Without establishing hydrogen bonds
  - In Van der Waals contact with several hydrophobic residues

↓pKa -> required for mechanism

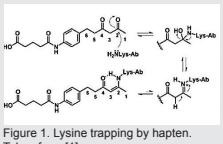

Figure 1. Lysine trapping by hapten. Taken from [1]

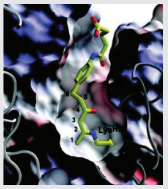

Figure 2. 32F12 active site. The enamine conjugate between LysH93 and hapten from Scheme 1 can be seen. Taken from [1]

4. Humanization process

❖ Required for feasibility of clinical trials

All mouse 38C2 (m38C2) CDR residues (from light chain and heavy chain) and 12 selected framework region (FR) residues were engrafted into a combination of human V and J genes for the light and heavy chain variable domains -> fusion to constant domains -> Fab fragment -> IgG

Selection of FR residues was based on 33F12 structure (see Fig.2):
-Catalytic lysine
-6 residues within 5A of LysH93 ε-amino group
-5 residues that line the substrate binding pocket

Humanized 38C2 (h38C2) had similar K_{cat} and k_M and preferred tertiary aldols compared to secondary aldols like m38C2 does.

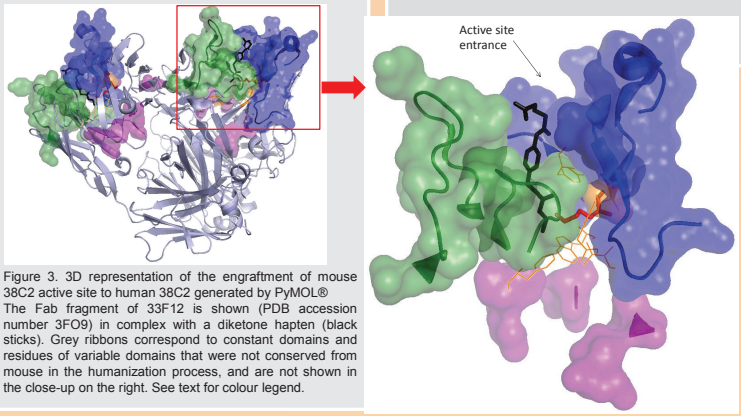

Figure 3. 3D representation of the engraftment of mouse 38C2 active site to human 38C2 generated by PyMOL®
The Fab fragment of 33F12 is shown (PDB accession number 3FO9) in complex with a diketone hapten (black sticks). Grey ribbons correspond to constant domains and residues of variable domains that were not conserved from mouse in the humanization process, and are not shown in the close-up on the right. See text for colour legend.

5. 38C2-based therapeutic strategies

Antibody-Directed Abzyme Prodrug Therapy (ADAPT)

Bifunctional antibodies { target-specific antibody; 38C2 for prodrug activation

Prodrugs contain a tertiary aldol and an oxa-Michael motif -> retro-aldol-retro-Michael tandem reaction by 38C2 releases free drug:

Figure 4. 38C2-catalysed prodrug activation. Adapted from [2]

This strategy achieves:
-Local targeted release of drug -> less side effects
-Specificity: hydrophobic prodrug is not a substrate for endogenous aldolases
-Less immunogenicity than an enzyme

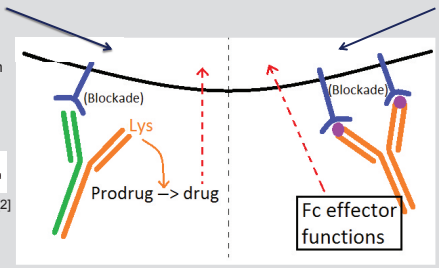

Figure 5. ADAPT and cpAbs modes of action
On the left, green corresponds to the target-specific arm and orange to the 38C2 catalytic arm of a bifunctional antibody for ADAPT.
On the right, orange represents 38C2 and purple a targeting agent. Membrane targets are shown in blue.

Chemically programmed antibodies (cpAbs)

- "Targeting agents" (TA) are attached to 38C2 through the reactive lysine LysH93 via:
  - Diketone compound
  - Vinylketone compound
  - βlactam group
- A linker is required to allow the TA to be exposed outside the 11 Å-deep active site
- Benefits:
  -Blood half-life of the TA is extended to that of an antibody
  -Effector functions of the antibody's Fc can contribute to the therapeutic action

❖ **Catalytic activity is lost!**
*This is used to verify successful conjugation

6. CpAbs for infectious disease treatment

The conjugation strategy is based on the reactivity of a β-lactam group with LysH93:

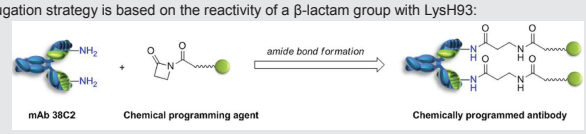

Figure 6. Taken from [3]

Influenza

- Zanamivir is a neuraminidase (NA) inhibitor -> blocks *de novo* virion release
- Half-life of 38C2-zanamivir cpAb was 120 hours (vs. 10 minutes for zanamivir)
  *2 doses / day -> 1/2 doses / month

❖ There is large antigenic variation on the surface of NA (for immune evasion). The active site of NA is less tolerant to mutations and can be reached by a small compound such as Zanamivir -> the cpAb approach combines the positive features of zanamivir and the antibody scaffold

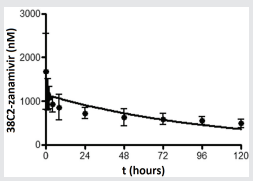

Figure 7. Blood half-life of zanamivir derived cpAb. Adapted from [3]

HIV-1

- Entrance of virions is mediated by gp120 antigen, which binds the CD4 receptor and CCR5 coreceptor
- CpAb of BMS-488043 (anti-gp120) has reduced but significant neutralization activity
- Maraviroc blocks CCR5.
  - CpAb retains full activity for 10 days
  - CpAb has broad spectra like Maraviroc (see Figure 7)
  - Unknown toxicity of cpAb

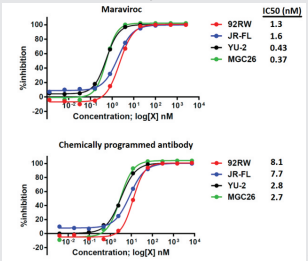

Figure 8. Neutralization assays using Maraviroc and corresponding cpAb. 92RW belongs to clade A, JR-FL and YU-2 to clade B and MGC26 to clade C.
Adapted from [4]

7. 38C2-based cancer treatment

ADAPT therapy

Prodrugs substrate for 38C2 have been generated from:
- Doxorubicin and duocarmycin (DNA intercalating agents)
- Etoposide and camptothecin (topoisomerase inhibitors)
- Enediynes (DNA-cleaving agents)

There is one *in vivo* study in mice with NXS2 cell line primary neuroblastomas:
- m38C2 (intratumoural) + etoposide prodrug (systemical) at 1250 mg/kg body weight -> 75% ↓ of tumor growth
- No side effects

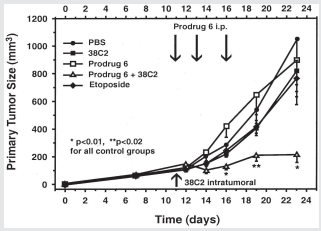

Figure 9. Taken from [5]

❖ The targeting arm of the bifunctional designed construct remains unexplored -> results have to be mainly attributed to the Enhanced Permeation Effect
❖ A variant of ADAPT was proposed: chemically labelled antibodies -> 38C2 + TA NOT attached to reactive lysine

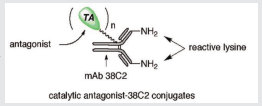

Figure 10. Taken from [6]

CpAbs

Integrin-targeted:
- TA: small integrin inhibitors
- Integrins are key regulators of angiogenesis
- CpAbs targeting integrins αvβ3 and αvβ5 can inhibit tumour growth by acting upon:
  - tumour cells. Figure 11: neoangiogenesis is not required for tumour development in the lung
  - endothelial cells
  - both

CovX-2000 platform
- Developed by Pfizer using h38C2
- TA are attached using an azetidinone linker (β-lactam approach)
- In phase I studies, all CovX-bodies (see Table 1) were well tolerated and allowed once a week dosing

❖ However, these cpAbs have been discontinued from Pfizer pipeline in 2014 -> uncertainty regarding further clinical trials

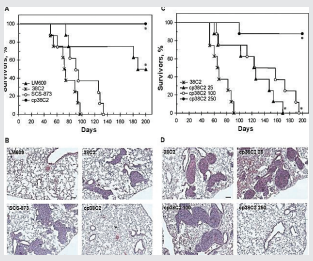

Figure 11. Lung metastatic foci in SCID mice inoculated with human melanoma M21 cells. LM609 binds human αvβ3 integrin. Doses correspond to μg / injection. Taken from [7]

Table 1. Summary of clinical trials with CovX-Bodies for cancer treatment. Adapted from [8]

CovX-Body	Mechanism of action	Clinical trials (phase; state)
CVX-060	Neutralize angiopoietin-2	NCT00879684 (phase I; completed) NCT00982657 (phase I/II; completed, no results available) *CVX-060 + sunitinib (tyrosine kinase inhibitor) NCT01441414 (phase II; terminated due to unexpected arterial and venous thrombotic events) *CVX-060 + axitinib (tyrosine kinase inhibitor)
CVX-045	Mimic thrombospondin-1	NCT00879554 (phase I; completed)
CVX-241	Neutralize angiopoietin-2 and VEGF	NCT01004822 (phase I; terminated due to poor pharmacokinetics, no safety concerns)

8. Conclusions

Catalytic antibody aldolase 38C2 is an excellent example of interdisciplinary transfer of knowledge because:

•. Detailed mechanistic information was used for the hapten design and trapping of the reactive lysine
•. Structural information was the basis for the successful humanization process
•. In spite of catalytic activity being lost, use of 38C2 as a chemically programmed antibody is a very promising and versatile therapeutic strategy that has reached clinical trials

9. Bibliography

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