Novel targeting strategies using recombinant antibodies for early diagnosis and therapy of ovarian cancer

"...carefully designed screening strategies of recombinant antibody libraries have the potential to lead to the identification of ideal targeting reagents for both in vitro *and* in vivo *uses."*

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Despite major efforts to identify biomarkers or biomarker panels performing better than CA125, which is the most studied ovarian cancer biomarker, no diagnostic marker for the early detection of ovarian cancer has been approved to date. Almost 30 years after the discovery of CA125 [1], ovarian cancer continues to be generally diagnosed at an advanced stage where the case:fatality ratio is high, thus remaining the most lethal of all gynecologic malignancies among US women. Attempts at screening with conventional imaging strategies have been hampered by high levels of false-positives with only 13–21% of the women that undergo surgery to remove adnexal masses actually having ovarian cancer [2]. As a result, while the case for early detection of ovarian cancer is undeniable [3], the current opinions regarding screening for ovarian cancer in the general population are divided between negative [4–6] and mildly positive [7,8] recommendations that emphasize stable markers in healthy controls over time. Clearly, to become a standard-of-care screening, modalities must be improved, but doubts about the achievability of the task haunt the field. When necessary to defy apparently grim probabilities, the quote of my compatriots, "Impossible n'est pas français," naturally comes to mind. ("Impossible n'est pas français" can be translated in English as "There is no such word as can't". This sentence attributed to Napoleon is actually a pun meaning both 'impossible is not French' and 'impossible is not idiomatically correct'.) Indeed, with the accelerated development of targeted macromolecules engineered for cargo delivery, early diagnosis and therapy of ovarian cancer now appear as a tangible future.

Macromolecules preferentially accumulate in tumors due to the enhanced permeability and retention effect that arises from the difference in clearance rate between solid tumors and normal tissues [9]. Various imaging and therapeutic reagents or reporters for molecular and functional imaging studies, ranging from lipid-based or paramagnetic nanoparticles to highly versatile polymers and viral capsids, can be used to detect and characterize pathological abnormalities [10–14]. The extra- or intracellular localization of the macromolecules can be controlled by coupling them to cell-penetrating peptides and transduction domains [15,16], while sensitivity and specificity can be increased by strategies such as attachment to proteins and particles, encapsulation into micelles and caged structures, biochemical reporters that are sensitive to pH, proteins, temperature or oxygen [17], and targeting to receptors. The conjugation of targeting platforms to nanoparticles optimizes specific local delivery. Any moiety with specific binding properties, such as monoclonal antibodies [18], or molecules that target cell receptors [19–21] can be used as targeting reagents. For example, the folate receptor-α that is highly expressed by ovarian cancer cells can be targeted by folate or by an antifolate receptor-α antibody. Yet, since antibodies can specifically bind to a unique antifolate receptor while folate binds to any folate receptors, the exquisite epitope recognition of antibody conceptually guarantees a much higher specificity than natural molecules. However, multiple hurdles – for which cost and time are not the least factors – exist on the road of production of monoclonal antibodies tolerated by the human immune system. Even if technical obstacles can be overcome given enough time and work, impediments linked to the immunization process cannot.

Antibodies are naturally produced by organisms in contact with foreign and immunogenic molecules, two characteristics that most tumor-associated antigens lack. Tumor-associated antigens are mainly endogenous molecules overexpressed by tumors rather than being tumor specific, and are often conserved between humans and animals used for immunization. In addition, the ideal epitopes for *in vivo* targeting cell-surfaced, overexpressed molecules are most

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likely conformational, but the engineering of recombinant molecules bearing conformational epitopes remains challenging. Finally, most tumor-associated antigens expressed by tumor cells display altered post-translational modifications, including glycosylation [22], which are both difficult to reproduce *in vitro* and poorly immunogenic. Consequently, instead of mounting an immune response against conformational epitopes or post-translational modifications uniquely expressed by tumor-associated antigens, immunized animals preferentially develop antibodies directed against the nonconserved regions of the recombinant proteins or linear peptides used for immunization. This can yield production of antibodies that do not cross-react with the corresponding tumor-associated antigen despite their high affinity for the immunizing agent. Thus, animal immunization does not permit the production of affinity reagents specifically optimized for cancer targeting. Furthermore, antibodies produced in animals are often immunogenic themselves in humans and, thus, require additional molecular re-engineering ('humanization') for safe clinical use. Finally, the absence of mouse antibody cross-reactivity with mouse antigens requires performing preclinical experiments in human xenograft mouse models, where it is impossible to assess the potentially harmful side effects caused by antibody offsite (i.e., nontumor) binding. In conclusion, although natural antibodies have proved invaluable in the diagnosis and treatment of infectious and autoimmune diseases, their use for cancer diagnosis or treatment may generate detection assays unable to distinguish between cases and controls, and produce offsite effects that cannot be addressed with the current preclinical models.

The identification of targeting reagents by screening recombinant antibody libraries circumvents caveats linked to immunization. Libraries of recombinant antibodies are produced by randomly recombining the hypervariable regions of the heavy and light immunoglobulin chains naturally produced by B lymphocytes or synthesized *in vitro* [23,24], and linking them together with [25] or without addition of constant domains to build Fab or scFv, respectively. More recently, novel classes of very small recombinant antibodies have been developed using only one domain derived from camelid immunoglobulin hypervariable regions [26], or from synthetic hypervariable regions [27]. Recombinant antibody libraries displayed by phage [28] or eukaryotes, such as yeast [29], can be screened by immunogenic, nonimmunogenic, native or denatured molecules, cell lines or tissues, *in vitro* or *in vivo* [30]. As the screening of recombinant antibody libraries identifies the best specific binders from a pool of existing molecules without any *de novo* generation through immunization, the successful isolation of effective affinity reagents only depends on the library size and diversity. The screening process can involve several rounds of selection, including the use of both positive and negative selection, to permit the isolation of affinity reagents highly specific for tumor-associated antigens. For example, the positive screening of recombinant antibodies that bind to tumor cells expressing tumor-associated antigens and that cross-react between mouse and human antigens can be followed by the negative screening of the selected recombinant antibodies that bind to similar antigens presented by normal cells. It is worth noting that recombinant antibodies derived from human B lymphocytes do not require the additional step of humanization for safe use in the clinic, as they display little or no immunogenicity. Altogether, carefully designed screening strategies of recombinant antibody libraries have the potential to lead to the identification of ideal targeting reagents for both *in vitro* and *in vivo* uses.

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Recombinant antibodies can be further tailored for specific applications using random evolution to increase affinity, molecular design to optimize *in vivo* stability by adding disulfide bonds or constant domains, or fusion with various structures for functionalization. For example, T cells transduced with viruses encoding cell-surface-expressed recombinant antibodies fused to the CD28/TCR-ζ chimeric signaling domain can be specifically activated after antigen binding and can kill tumor cells [31]. Alternatively, recombinant antibodies can be genetically or chemically fused with bacterial or plant toxins for use as immunotoxins [32]. The fusion of recombinant antibodies to detection reagents, toxins or nanoparticles is crucial for both *in vitro* diagnostic tests as well as for *in vivo* targeted-imaging or targeted-therapy strategies. However, challenges associated with the fusion steps are often underestimated. Specific requirements must be met, such as the generation of a

stable bond under a wide range of conditions that does not compromise antigen-binding function, and that is not immunogenic for *in vivo* applications. At this time, no method yields predictable results. Chemical covalent bonding remains the prevalent method to fuse recombinant antibodies with functional domains or nanoparticles, but it needs to be optimized on a case-by-case basis as it produces conformational changes that can negatively impact antigen recognition. For *in vitro* applications the use of avidin/biotin as a scaffold, including short biotin acceptor tags fused to the recombinant antibodies [33–36], is a practical and highly efficient method. However, even though streptavidin has been used for pretargeted radioimmunotherapy [37] and methods to lower streptavidin immunogenicity are available [38,39], the appearance of human antistreptavidin antibodies in 60–80% of patients exposed to avidin or streptavidin [40] is concerning. Alternative methods of coupling need to be developed for *in vivo* usage. We believe that enzymatic cross-linking using transglutaminases [41] offers a powerful potential for this specific application. Transglutaminases encompass a group of enzymes found in various tissues, including skin and hair, as well as in blood clotting and wound healing, with the ability to act as biological glues by catalyzing protein post-translational modifications (isopeptide bonds) either through cross-linking via ε-(γ-glutamyl) lysine bonds or through incorporation of primary amines at selected peptide-bound glutamine residues. Thus, transglutaminases are able to mediate enzymatic cross-linking that does not form any immunogenic structures and does not affect antigen recognition.

In conclusion, recent experimental advances have increased our confidence in the screening of large recombinant antibody libraries to isolate and design optimized targeting reagents for both *in vitro* and *in vivo* early diagnosis and treatment of ovarian cancer. Collaborations between immunologists and bioengineers should be strongly encouraged and supported, as they have the unique potential to generate the tools necessary to diagnose ovarian cancer at an early stage when it is still treatable, and to monitor and prevent cancer relapses, in the near future.

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Bibliography

1 Bast RC Jr, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC: Reactivity of a monoclonal antibody with human ovarian carcinoma. *J. Clin. Invest.* 68(5), 1331–1337 (1981).

2 Shaaban A, Rezvani M: Ovarian cancer: detection and radiologic staging. *Clin. Obstet. Gynecol.* 52(1), 73–93 (2009).

3 Etzioni R, Urban N, Ramsey S *et al.*: The case for early detection. *Nat. Rev. Cancer.* 3(4), 243–252 (2003).

4 Partridge E, Kreimer AR, Greenlee RT *et al.*: Results from four rounds of ovarian cancer screening in a randomized trial. *Obstet. Gynecol.* 113(4), 775–782 (2009).

5 Clarke-Pearson DL: Clinical practice. Screening for ovarian cancer. *N. Engl. J. Med.* 361(2), 170–177 (2009).

6 Mutch DG: Ovarian cancer: to screen or not to screen. *Obstet. Gynecol.* 113(4), 772–774 (2009).

7 Menon U, Gentry-Maharaj A, Hallett R *et al.*: Sensitivity and specificity of multimodal and ultrasound screening for ovarian cancer, and stage distribution of detected cancers: results of the prevalence screen of the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). *Lancet Oncol.* 10(4), 327–340 (2009).

8 Anderson GL, Mcintosh M, Wu L *et al.*: Assessing lead time of selected ovarian cancer biomarkers: a nested case–control study. *J. Natl Cancer Inst.* 102, 26–38 (2010).

9 Noguchi Y, Wu J, Duncan R *et al.*: Early phase tumor accumulation of macromolecules: a great difference in clearance rate between tumor and normal tissues. *Jpn. J. Cancer Res.* 89(3), 307–314 (1998).

10 Zheng G, Chen J, Li H, Glickson JD: Rerouting lipoprotein nanoparticles to selected alternate receptors for the targeted delivery of cancer diagnostic and therapeutic agents. *Proc. Natl Acad. Sci. USA* 102(49), 17757–17762 (2005).

11 Myhr G: MR guided cancer treatment system for an elevated therapeutic index – a macroscopic approach. *Med. Hypotheses* 70(3), 665–670 (2008).

12 Green JJ, Langer R, Anderson DG: A combinatorial polymer library approach yields insight into nonviral gene delivery. *Acc. Chem. Res.* (2008) (Epub ahead of print).

13 Bilensoy E, Hincal AA: Recent advances and future directions in amphiphilic cyclodextrin nanoparticles. *Expert Opin. Drug Deliv.* 6(11), 1161–1173 (2009).

14 Steinmetz NF, Lomonossoff GP, Evans DJ: Cowpea mosaic virus for material fabrication: addressable carboxylate groups on a programmable nanoscaffold. *Langmuir* 22(8), 3488–3490 (2006).

15 Bhorade R, Weissleder R, Nakakoshi T, Moore A, Tung CH: Macrocyclic chelators with paramagnetic cations are internalized into mammalian cells via a HIV-Tat derived membrane translocation peptide. *Bioconjug. Chem.* 11(3), 301–305 (2000).

16 Allen MJ, Macrenaris KW, Venkatasubramanian PN, Meade TJ: Cellular delivery of MRI contrast agents. *Chem. Biol.* 11(3), 301–307 (2004).

17 Yoo B, Pagel MD: An overview of responsive MRI contrast agents for molecular imaging. *Front. Biosci.* 13, 1733–1752 (2008).

18 Artemov D, Mori N, Ravi R, Bhujwalla ZM: Magnetic resonance molecular imaging of the HER-2/Neu receptor. *Cancer Res.* 63(11), 2723–2727 (2003).

19 Gustafsson B, Youens S, Louie AY: Development of contrast agents targeted to macrophage scavenger receptors for MRI of vascular inflammation. *Bioconjug. Chem.* 17(2), 538–547 (2006).

20 Major JL, Meade TJ: Bioresponsive, cell-penetrating, and multimeric MR contrast agents. *Acc. Chem. Res.* 42(7), 893–903 (2009).

21 Hattori Y, Maitani Y: Folate-linked lipid-based nanoparticle for targeted gene delivery. *Curr. Drug Deliv.* 2(3), 243–252 (2005).

22 Dennis JW: *N*-linked oligosaccharide processing and tumor cell biology. *Semin. Cancer Biol.* 2(6), 411–420 (1991).

23 Ward ES, Gussow D, Griffiths AD, Jones PT, Winter G: Binding activities of a repertoire of single immunoglobulin variable domains secreted from *Escherichia coli*. *Nature* 341(6242), 544–546 (1989).

24 Ohara R, Knappik A, Shimada K, Frisch C, Ylera F, Koga H: Antibodies for proteomic research: comparison of traditional immunization with recombinant antibody technology. *Proteomics* 6(9), 2638–2646 (2006).

25 Hust M, Jostock T, Menzel C *et al.*: Single chain Fab (scFab) fragment. *BMC Biotechnol.* 7, 14 (2007).

26 Harmsen MM, Ruuls RC, Nijman IJ, Niewold TA, Frenken LG, De Geus B: Llama heavy-chain V regions consist of at least four distinct subfamilies revealing novel sequence features. *Mol. Immunol.* 37(10), 579–590 (2000).

27 Sidhu SS, Li B, Chen Y, Fellouse FA, Eigenbrot C, Fuh G: Phage-displayed antibody libraries of synthetic heavy chain complementarity determining regions. *J. Mol. Biol.* 338(2), 299–310 (2004).

28 Clackson T, Hoogenboom HR, Griffiths AD, Winter G: Making antibody fragments using phage display libraries. *Nature* 352(6336), 624–628 (1991).

29 Feldhaus MJ, Siegel RW, Opresko LK *et al.*: Flow-cytometric isolation of human antibodies from a nonimmune *Saccharomyces cerevisiae* surface display library. *Nat. Biotechnol.* 21(2), 163–170 (2003).

30 Pasqualini R, Ruoslahti E: Organ targeting *in vivo* using phage display peptide libraries. *Nature* 380(6572), 364–366 (1996).

31 Haynes NM, Trapani JA, Teng MW *et al.*: Single-chain antigen recognition receptors that costimulate potent rejection of established experimental tumors. *Blood* 100(9), 3155–3163 (2002).

32 Mathew M, Verma RS: Humanized immunotoxins: a new generation of immunotoxins for targeted cancer therapy. *Cancer Sci.* 100(8), 1359–1365 (2009).

33 Beckett D, Kovaleva E, Schatz PJ: A minimal peptide substrate in biotin holoenzyme synthetase-catalyzed biotinylation. *Protein Sci.* 8(4), 921–929 (1999).

34 Scholler N, Garvik B, Quarles T, Jiang S, Urban N: Method for generation of *in vivo* biotinylated recombinant antibodies by yeast mating. *J. Immunol. Methods* 317(1–2), 132–143 (2006).

35 Bellin MF, van der Molen AJ: Extracellular gadolinium-based contrast media: an overview. *Eur. J. Radiol.* 66(2), 160–167 (2008).

36 Valadon P, Darsow B, Buss TN *et al.*: Designed auto-assembly of nanostreptabodies for rapid tissue-specific targeting *in vivo*. *J. Biol. Chem.* 285(1), 713–722

37 Goldenberg DM, Sharkey RM, Paganelli G, Barbet J, Chatal JF: Antibody pretargeting advances cancer radioimmunodetection and radioimmunotherapy. *J. Clin. Oncol.* 24(5), 823–834 (2006).

38 Chinol M, Casalini P, Maggiolo M *et al.*: Biochemical modifications of avidin improve pharmacokinetics and biodistribution, and reduce immunogenicity. *Br. J. Cancer* 78(2), 189–197 (1998).

39 Jones TD, Crompton LJ, Carr FJ, Baker MP: Deimmunization of monoclonal antibodies. *Methods Mol. Biol.* 525, 405–423, xiv (2009).

40 Chinol M, Grana C, Gennari R, Cremonesi M, Geraghty JG, Paganelli G: Pretargeted radioimmunotherapy of cancer. In: *Radioimmunotherapy of Cancer.* Abrams PG, Fritzberg AR (Eds). Informa Healthcare, NY, USA (2000).

41 Griffin M, Casadio R, Bergamini CM: Transglutaminases: nature's biological glues. *Biochem. J.* 368(Pt 2), 377–396 (2002).