EXPERT OPINION ON BIOLOGICAL THERAPY
https://doi.org/10.1080/14712598.2020.1696767

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EDITORIAL

Rationale, discovery and clinical development of NEO-201

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ARTICLE HISTORY Received 11 June 2019; Accepted 20 November 2019

KEYWORDS CEACAMs; immunotherapy; monoclonal antibody; NEO-201

1. Introduction

Most monoclonal antibody cancer therapies are developed for known targets and are commercially approved for the treatment of a broad spectrum of cancers; however, the side effects can be severe because many of their respective targets are located on tumor cells and normal, healthy cells.

We have taken a different approach to the development of potential therapeutic antibodies for cancer. Instead of using known antigens or finding new targets using cancer cell lines, we have taken a reverse approach. We utilized immunogenic cell membrane extracts derived from pooled allogeneic tumors acquired at the time of surgery. This extract, used as a cancer vaccine in clinical trials, demonstrated antitumor activity. There was a direct correlation between IgG responses to the vaccine and tumor regression and overall survival. This vaccine was used as a platform to screen for monoclonal antibodies with tumor sensitivity and specificity. Targets were identified using the selected antibodies for immunoprecipitation of antigens and mass spectrometry. We then identified the actual targets of these functional antibodies.

2. Development of a new monoclonal antibody: the NEO-201 story

NEO-201 is one of several IgG1 mAbs that were generated against an allogeneic colorectal cancer vaccine platform [1,2]. In order to produce a potentially effective vaccine, the investigators attempted to isolate immunogenic proteins that could be tested for both function and immunogenicity. The immunogenic components of this vaccine were TAAs that were derived from tumor membrane fractions pooled from surgically resected specimens from 79 patients with colon cancer [3]. Two of these extracts, when injected subcutaneously, produced delayed type hypersensitivity (DTH) reactions in colorectal cancer patients but were unable to elicit a DTH response in a cohort of healthy volunteers. This material was used to create an allogenic tumor vaccine that was administered in a clinical trial to patients with chemotherapy-resistant colorectal cancer. A direct correlation was observed between development of antitumor response and the ability to mount and sustain high levels of IgG post-vaccination [4]. We used this vaccine to screen for antibodies that were both sensitive and specific to colon cancer, sequencing their CDR regions and creating humanized IgG1 subtypes. These monoclonal antibodies were initially screened against colorectal cancer cell lines and then screened for their ability to lyse human colon cancer. This process yielded the previously described ensituximab (NPC-1C/NEO-102) [5–7] and NEO-201, both of which were able to destroy colon cancer cells in vitro through the mechanism of antibody-dependent cell cytotoxicity (ADCC). Since NEO-201 was produced against antigens in the allogeneic cancer vaccine that had not been previously identified, additional studies were performed to identify the target for this antibody. Through immunoprecipitation and mass spectrometry, NEO-201 was found to bind tumor-associated variants of CEACAM family members, particularly cancer-associated variants of CEACAM5 and CEACAM6 [8].

3. NEO-201 preclinical testing

CEACAM family proteins are expressed by different epithelial tissues, and post-transcriptional modifications to these proteins have been found to be related to tumorigenesis and metastasis, making CEACAM an appealing target for drug development [9]. Several antibodies targeting CEACAMs are now available, but none has showed direct anti-tumor activity, and some have been used for drug-conjugated antibodies [10]. The limited specificity for cancer tissue and the cross-reactivity with the surrounding healthy tissue has limited the progress in this field. When compared with the anti-CEACAM-6 and anti-CEACAM-5 antibodies 9A6 and CB30, NEO-201 showed a high sensitivity and specificity for cancer tissue but not for surrounding healthy tissue (Figure 1) [11]. Among the tumor types analyzed, the NEO-201 epitope was highly expressed not only in colon and pancreatic cancer, but also breast, lung, and ovarian mucinous carcinoma. This data suggests that NEO-201 might be useful as a backbone for drug-conjugated antibodies and chimeric antigen receptor T (CAR-T) cell therapy [12].

Although CEACAM family proteins have no proven role in tumor growth [13], the NEO-201 epitope is a trigger for direct anti-tumor activity. As previously described by our group, NEO-201 showed promising activity against pancreatic cancer models in vivo and in vitro [11] (see Figure 2). The antibody's anti-tumor activity was mostly observed in the presence of purified natural killer (NK) cells or purified complement factors, leading to the conclusion that ADCC and complement-dependent cytotoxicity

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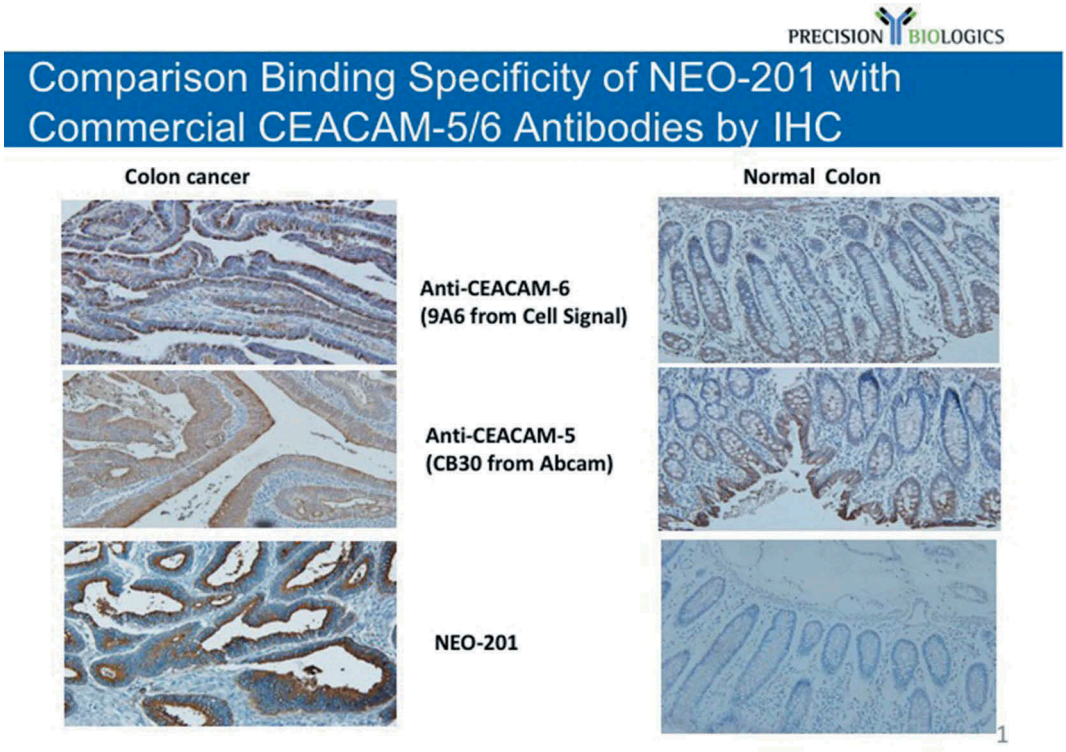

Figure 1. Sensitivity and specificity of NEO-201 for tumor tissue.

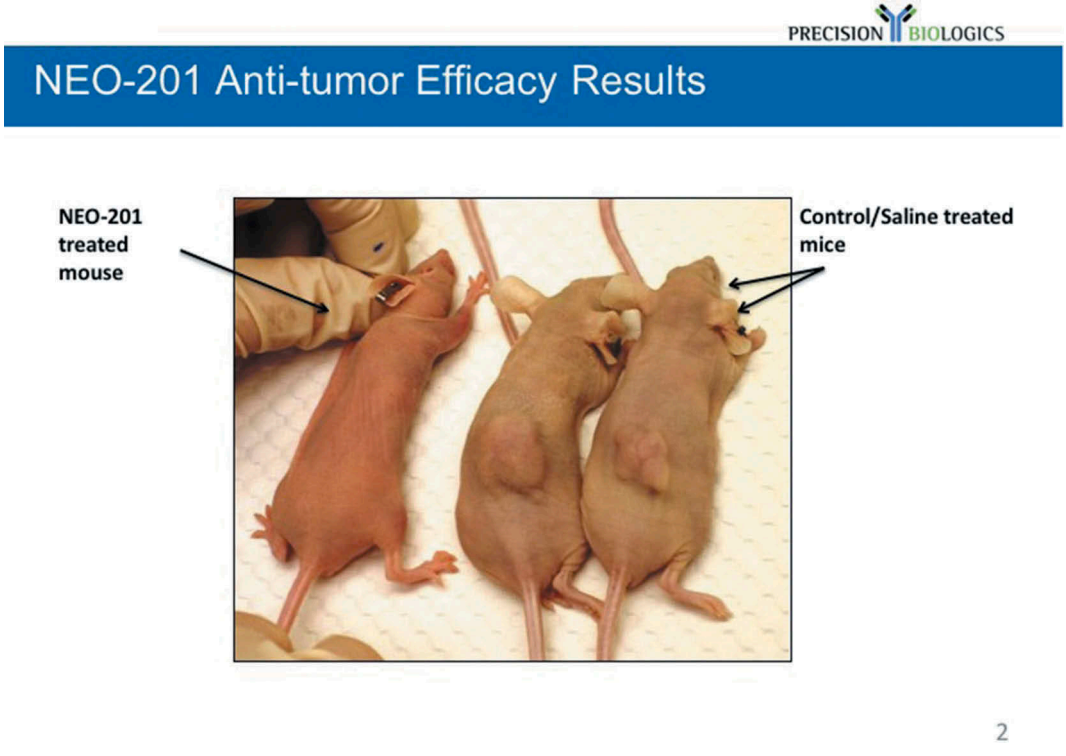

Figure 2. NEO-201 anti-tumor activity in a pancreatic cancer model.

are the mechanisms behind the direct anti-tumor effect of NEO-201. Tissue cross-reactivity studies of NEO-201 showed cross-reactivity with human granulocytes due to their expression of CEACAM-6, and, among the mammalian species tested, only the granulocytes from the non-human primates were positive for the NEO-201 epitope; therefore, they were chosen as the most relevant animal species for pre-clinical pharmacology studies. In order to move to clinical testing, safety data were obtained from both single and multi-dose studies on cynomolgus monkeys [11]. Overall treatment was well tolerated across the three

dose levels evaluated (4 mg/kg, 20 mg/kg, and 49 mg/kg), and the most common side effect observed was transient neutropenia [11]. The neutropenia occurred in all 3 groups occurred within 2 days of receiving the antibody, and neutrophils generally recovered to at least 80% of baseline within a week of treatment. The decline in neutrophil counts varied in individual animals. This ranged from grade 1, with counts at lower levels of the normal limit to > 1500/mm^3, to grade 4, with neutrophil counts < 500/mm^3. This phenomenon was observed regardless of the dose of antibody tested. Neutrophil recovery was not diminished when multiple doses of antibody were administered. In addition, there was no sign of bone marrow suppression and no significant decline in serum levels of red blood cells or platelets.

4. NEO-201 clinical development

Currently, a first-in-human phase I clinical trial is ongoing at the NCI to evaluate the safety and tolerability of NEO-201 in patients with solid tumors. The study design is a classic Fibonacci (3 + 3) dose-escalation trial, with an expansion cohort to better evaluate the dose selection for phase II trials and identify specific patient populations. During the dose-escalation phase, ancillary studies will be conducted in the laboratory to identify biomarkers for patient selection. Although the IHC detection of the NEO-201 epitope seems to be a rational biomarker, a scoring system needs to be optimized and validated before making any conclusion.

Additionally, the role of level of circulating CEA as a possible biomarker and the relationship between tumor marker levels in the blood and response to treatment are unclear and need to be better understood. In the Phase I study, serum CEA levels are being collected at baseline and then every 2 cycles (4 doses) during treatment. This should help determine the significance of CEA as a serum biomarker of clinical activity of NEO-201. Once the recommended phase II dose is identified, biomarker-driven expansion cohorts will be opened to evaluate the activity of NEO-201 in selected patient populations.

5. Expert opinion

NEO-201 is a novel humanized IgG monoclonal antibody derived from an immunogenic cancer vaccine that has shown a specific reactivity against different epithelial tumors without reactivity toward the respective healthy tissues. The specificity of NEO-201 correlated with its binding to a specific tumor associated variant of CEACAM-5 and −6 expressed across different cancer subtypes. Interestingly, although this neo-epitope is not found on normal epithelial cells, it is found on normal hemopoietic cells, specifically granulocytes. This raises the question of whether the target would also be found in any hematologic malignancies in addition to solid tumors.

Unlike commercial antibodies that target CEACAM-5 and −6, our preclinical data suggest that NEO- 201 can trigger NK cell-mediated ADCC in human cancer cells and inhibit the growth of human tumor xenografts in mice (see Figure 2). Other CEA antibodies have been modified in the effort to produce antitumor effects. These have consisted of approaches such as developing CAR-T cells, developing antibody drug conjugates, or conjugating with a radiolabeled isotope. Although these modified antibodies have improved tumor-killing capabilities, it is important to monitor for untoward adverse events, including colitis, in the clinic.

Considering the complexity and heterogeneity of solid tumors, it is of concern that treatment with NEO-201 alone might not be enough to achieve a durable clinically relevant antitumor effect. Other combination approaches need to be evaluated in the laboratory and translated to the clinic if successful. The ADCC process is mainly related to the induction of phagocytosis and lysis of mAb-opsonized cancer cells by macrophages and NK cells, respectively. In a different study, we evaluated the effect of the cytokine interleukin-15 (IL-15) on ADCC mediated by NEO-201. This cytokine plays a crucial role in the development and activation of NK cells and was found to enhance ADCC against a wide range of human cancer cells expressing the NEO- 201 target. In our study, we showed that IL-15 modulates gene expression, inducing upregulation of factors involved in NK cytotoxicity, when administered with NEO-201 in vitro [8].

The toxicology safety studies performed in non-human primates demonstrated that NEO-201 was safe and well tolerated, with a transient decrease in circulating neutrophils being the only significant adverse effect observed. As noted, the levels ranged from Grade 1 to Grade 4 declines. It is well known that CEACAM-5 and −6 are expressed on granulocytes, and this can explain the neutropenia. Typically, neutropenia is an undesirable treatment side effect; however, in this scenario, it may actually be a marker of drug bioavailability and activity. Furthermore, neutropenia can often be treated fully with the use of G-CSF. The first-in-human phase I clinical trial that is currently ongoing at the NCI will further clarify the safety profile of the drug and the dose to further develop in the clinic. In addition, data from the trial may lead to the identification of possible biomarkers for patient selection with the ultimate goal of conducting a biomarker-driven phase II clinical trial.

Funding

This paper was funded by Precision Biologics.

Declaration of interest

PM Arlen is an employee of Precision Biologics and owns stock in the company. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

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