Stability Tests to Monitor the Shelf Life Of Monoclonal Antibodies Employed in Cancer Immunotherapy

MASSIMO FANTINI, PhD
Senior Scientist
PRECISION BIOLOGICS, INC.

6th Annual Neoantigen Based Therapies Summit
October 26th 2021

Forward Looking Statements

Statements in this Presentation that are not statements of historical fact are forward-looking statements. Such forward-looking statements include, without limitation, statements regarding our research and clinical development activities, plans and projected timelines, business strategy and plans, regulatory matters, objectives of management for future operations, market size and opportunity, our ability to complete certain milestones and our expectations regarding the relative benefits of our drug candidates versus competitive therapies. Words such as “believe,” “can”, “continue,” “anticipate,” “could,” “estimate,” “plan,” “predict,” “expect,” “intend,” “will,” “may,” “goal,” “upcoming,” “near term”, “milestone”, “potential,” “target” or the negative of these terms or similar expressions are intended to identify forward-looking statements, though not all forward-looking statements necessarily contain these identifying words. Because such statements are subject to risks and uncertainties, actual results may differ materially from those expressed or implied by such forward-looking statements. Risks that contribute to the uncertain nature of the forward-looking statements include, without limitation: our preclinical studies and clinical trials may not be successful; regulatory authorities, including the U.S. Food and Drug Administration (FDA) may not agree with our interpretation of the data from clinical trials of our drug candidates; we may decide, or regulatory authorities may require us, to conduct additional clinical trials or to modify our ongoing clinical trials; we may experience delays in the commencement, enrollment, completion or analysis of clinical testing for our drug candidates, or significant issues regarding the adequacy of our clinical trial designs or the execution of our clinical trials may arise, which could result in increased costs and delays, or limit our ability to obtain regulatory approval; our drug candidates may not receive regulatory approval or be successfully commercialized; unexpected adverse side effects or inadequate therapeutic efficacy of our drug candidates could delay or prevent regulatory approval or commercialization; the COVID-19 pandemic may disrupt our business and that of third parties on which we depend, including delaying or otherwise disrupting our research and development activities; and we may not be able to obtain additional financing. These forward-looking statements should not be taken as forecasts or promises nor should they be taken as implying any assurance or guarantee that the assumptions on which such forward-looking statements have been made are correct or exhaustive. You are cautioned not to place undue reliance on these forward-looking statements, which speak only as of the date this Presentation is given. Other risks and uncertainties affecting us are described more fully in our filings with the Securities and Exchange Commission. We undertake no obligation to update such statements to reflect events that occur or circumstances that exist after the date on which they were made.

This Presentation discusses drug candidates that are under clinical study and which have not yet been approved for marketing by the U.S. Food and Drug Administration. No representation is made as to the safety or effectiveness of these drug candidates for the use for which such drug candidates are being studied.

OUTLINE

- **OVERVIEW OF IMPORTANCE OF STABILITY TESTS FOR DRUGS EMPLOYED IN CLINIC**
- **MONOCLONAL ANTIBODIES EMPLOYED IN CANCER IMMUNOTHERAPY**
- **PRECISION BIOLOGICS MONOCLONAL ANTIBODY: NEO-201**
- **STABILITY TESTS USED TO TEST NEO-201 STABILITY AND BIOLOGICAL ACTIVITY**

PRECISION BIOLOGICS

SCOPE OF STABILITY TESTING

1) **To provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light**

2) **To establish a re-test period for the drug substance or a shelf life for the drug product and recommended storage conditions** (example 5°C upright, 5ºC inverted, -20ºC -60ºC)

PRECISION BIOLOGICS

STABILITY TESTING: DRUG SUBSTANCE vs DRUG PRODUCT

DRUG SUBSTANCE: is the *active ingredient* which is used to make the Drug Product

The Drug Substance provides the pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease.

DRUG PRODUCT: is the finished product of any drug that is available in the market and is ready to use

Drug product is the drug substance mixed with other components (for example excipients) which becomes available for use in the market.

STABILITY TESTING: DRUG SUBSTANCE

1) **STRESS TESTING:** can help identify the likely degradation products and intrinsic stability of the molecule. It include:

- **The effect of temperatures and humidity** (e.g., 75% RH or greater)
- **Oxidation and photolysis on the drug substance**
- **The susceptibility of the drug substance to hydrolysis** across a wide range of pH values when in solution or suspension.

Results from these studies will form an integral part of the information provided to regulatory authorities.

PRECISION BIOLOGICS

STABILITY TESTING: DRUG SUBSTANCE

2) **CONTAINER CLOSURE SYSTEM:** the stability studies should be conducted on the drug substance packaged in a container closure system that is the same as or simulates the packaging proposed for storage and distribution.

3) **SPECIFICATION:** stability studies should include testing of those attributes of the drug substance that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy, such as **physical, chemical, biological, and microbiological attributes.**

4) **STORAGE CONDITION**: a drug substance should be evaluated under storage conditions (with appropriate tolerances) that test its thermal stability and, if applicable, its sensitivity to moisture.

STABILITY TESTING: DRUG SUBSTANCE

TESTING FREQUENCY: For drug substances with a proposed re-test period of at least 12 months, the frequency of testing at the long-term storage condition should normally be:

- **Every 3 months over the first year**
- **Every 6 months over the second year**
- **Annually thereafter through the proposed re-test period.**

STABILITY TESTING: DRUG PRODUCT

The design of stability studies for the drug product should be based on knowledge of the behavior and properties of the drug substance and from stability studies on the drug substance

1) **PHOTOSTABILITY TESTING:** evaluation of the effect of the light on drug structure integrity

2) **CONTAINER CLOSURE SYSTEM:** dosage form packaged in the container closure system proposed for marketing (including, as appropriate, any secondary packaging and container label).

3) **SPECIFICATION**: include testing of those attributes of the drug product that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy, such as **the physical, chemical, biological, and microbiological attributes, preservative content** (e.g., antioxidant, antimicrobial preservative)

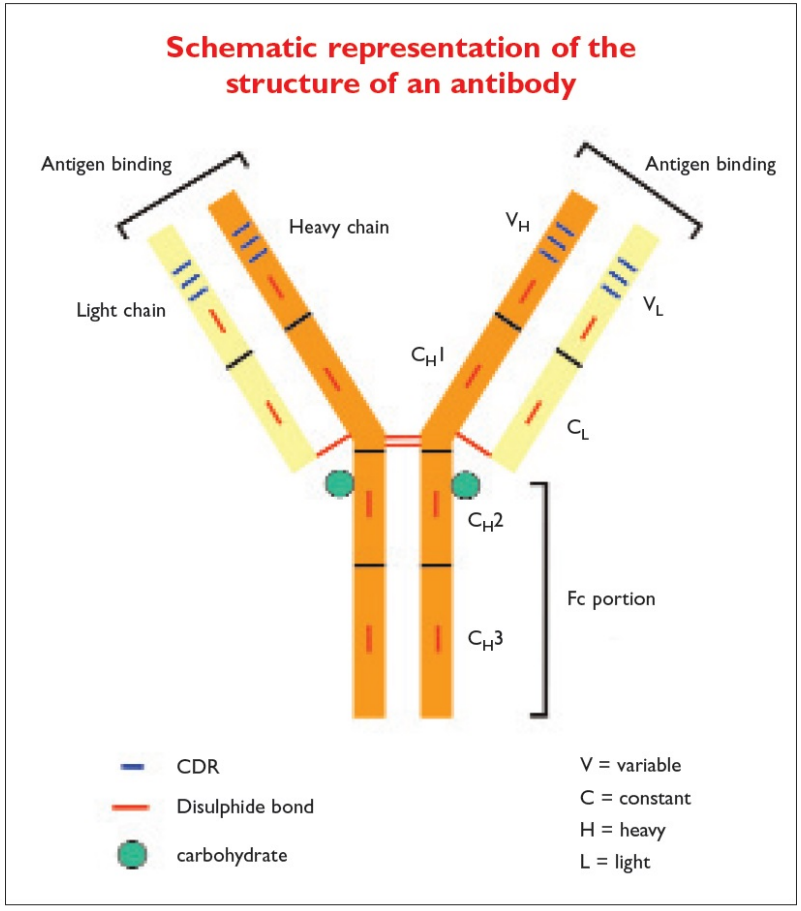
4) **STORAGE CONDITION**: tests its thermal stability, its sensitivity to moisture or potential for solvent loss.

STABILITY TESTING: DRUG PRODUCT

TESTING FREQUENCY: For products with a proposed shelf life of at least 12 months, the frequency of testing at the long-term storage condition should be:

- **Every 3 months over the first year**
- **Every 6 months over the second year**
- **Annually thereafter through the proposed shelf life.**

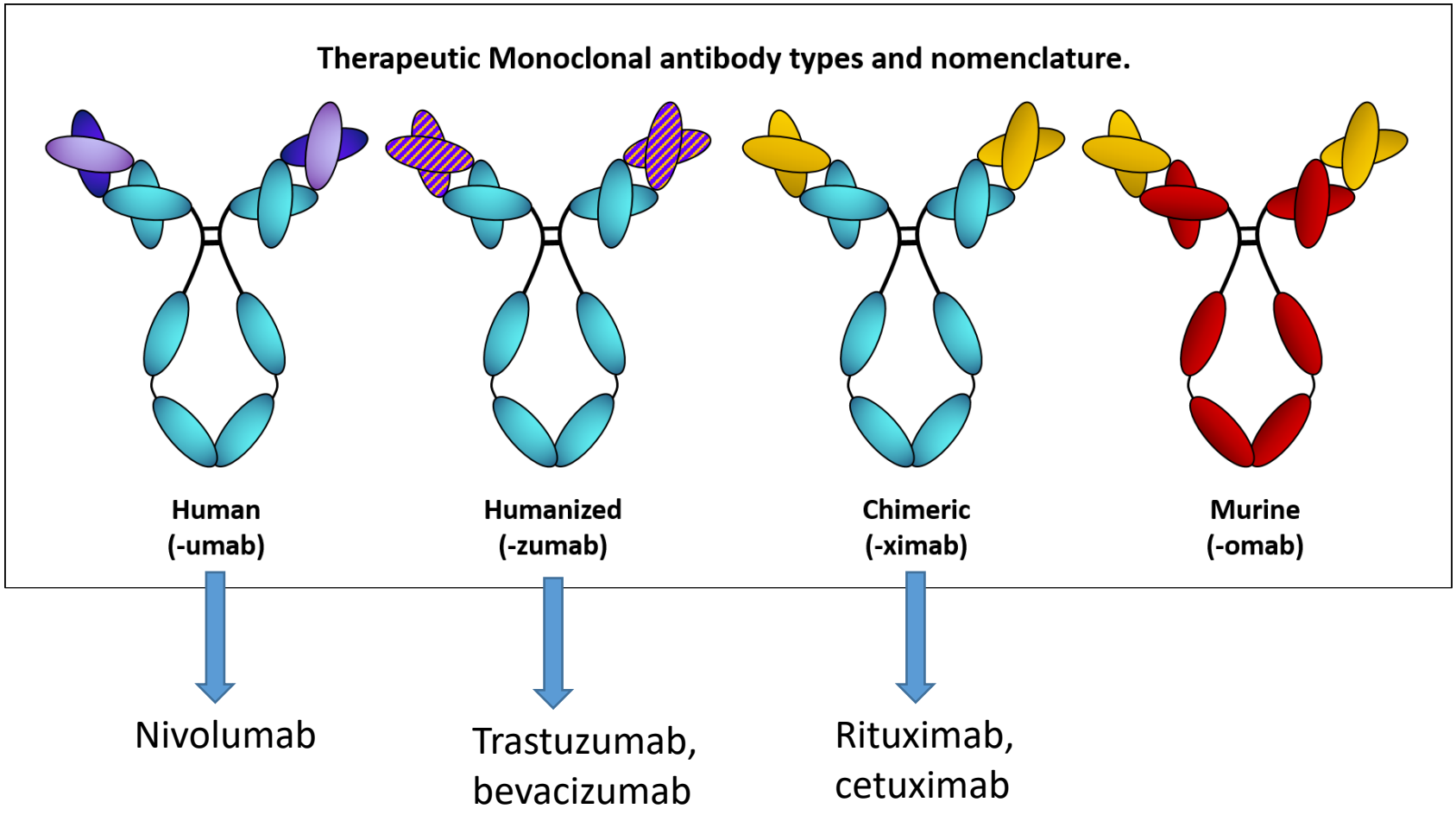
Monoclonal Antibodies Employed in Cancer Immunotherapy



https://www.ddw-online.com/monoclonal-antibodies-magic-bullets-or-a-shot-in-the-dark-1179-200208/

- Two identical **heavy chains** in association with two identical **light chains**
- **Variable region** binds to the target antigen through hypervariable loops called **CDRs (Complementary Determining Regions)**
- **Biological effector functions,** such as ADCC and CDC are mediated by the **Fc portion** of the antibody

Monoclonal Antibodies Employed in Cancer Immunotherapy

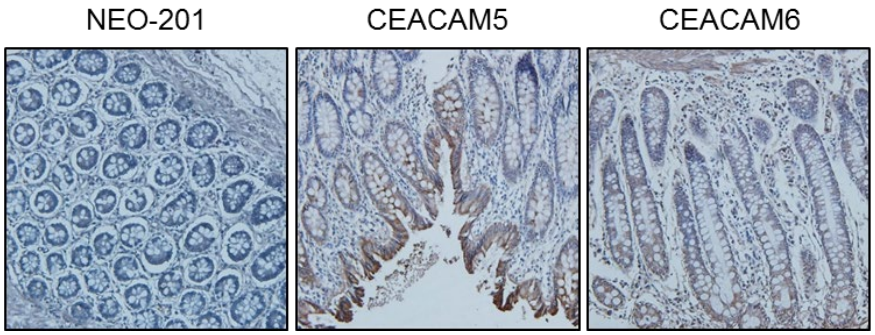


https://www.tebu-bio.com/blog/monoclonal-antibodies-all-you-need-to-know-about-antibody-generation/

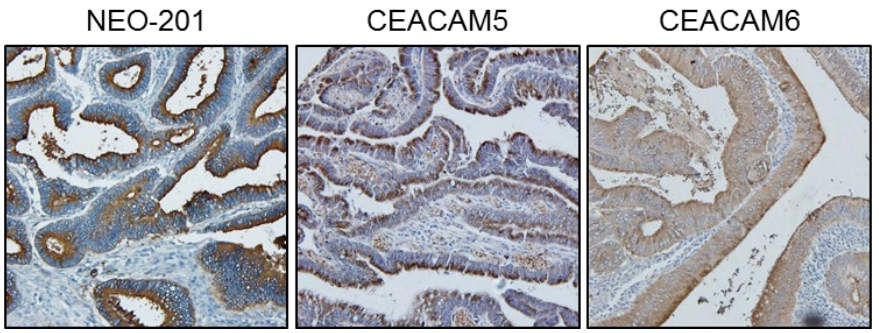
Stability Tests to Monitor the Shelf Life Of Monoclonal Antibodies Employed in Cancer Immunotherapy : experience with the Precision Biologic monoclonal antibody NEO-201

NEO-201 reactivity is tumor-associated

Normal Colon Tissue



Tumor Tissue



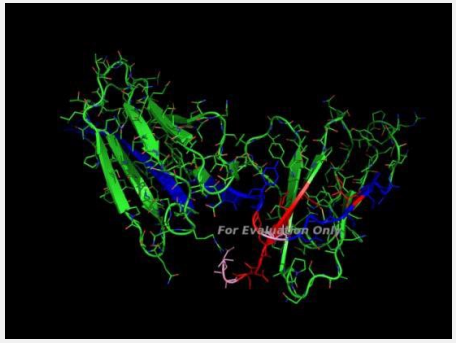
- NEO-201 does not react against normal epithelial tissue CEACAM5/6 positive.
  - Majority of normal tissues stained CEACAM5^{+} and/or CEACAM6^{+}
  - Colon (29/31, 94%), pancreatic (26/28, 93%), lung (30/32, 94%)

- NEO-201 reacts against tumor tissue CEACAM5/6 positive.
  - Majority of sampled tumors stained "triple positive" – NEO-201^{+} CEACAM5^{+} CEACAM6^{+}
  - Colon (28/32, 88%), pancreatic (23/30, 77%), lung (16/32, 50%)

NEO-201: Multiple Mechanisms of Action

Variant CEACAM-5, CEACAM-6 (cancer tissue-associated)

No NEO-201 Binding

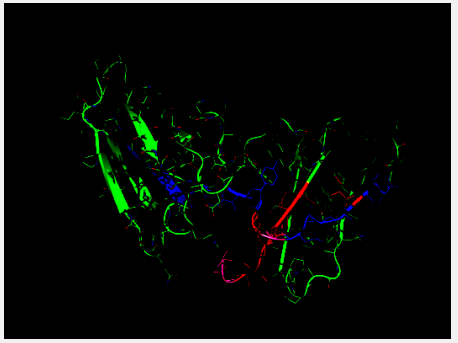


CEACAM-5/6
(healthy tissue)

NPC-1C antibodies

Protein target

NEO-201 Binding



Variant CEACAM-5/6
(cancer tissue-associated)

NEO-201: Direct Tumor Killing & Killing Through Immune Enhancement

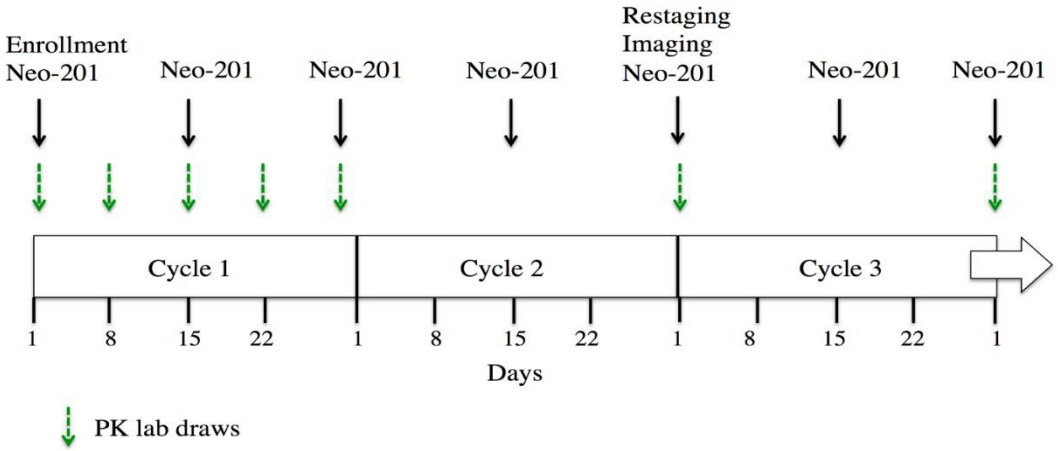
- Kills target through **Antibody dependent cell-mediated cytotoxicity (ADCC)** and **Complement dependent cytotoxicity (CDC)** unlike other CEA antibodies
- Enhanced NK tumor killing through CEACAM5/CEACAM1 blockade
- **Binding and killing of human regulatory T cells (Tregs) – supporting combination therapy with checkpoint therapy**

NEO-201 Completed Phase I Clinical Trial

Completed NEO-201, 1st in Human Clinical Trial

- NEO-201- 1st in human studies treated patients with colon ca, pancreatic ca, breast ca, NSCLC, and mucinous ovarian ca, who no longer have effective standard therapies available (either recurred, relapsed or progressed).
- Completed first in-human clinical studies at the NCI in patients with refractory solid tumors

Study Schema



PRECISION BIOLOGICS

Phase 1 Dose Escalation of NEO-201

Gender	Male: 6 (35%)	Female: 11 (65%)			
Age (years)	30-50: 5 (29%)	51-60: 4 (24%)	61-70: 5 (29%)	71-80: 2 (12%)	> 80: 1 (6%)
Cancer Type	Colorectal: 11 (65%)	Pancreatic: 4 (24%)	Breast: 2 (12%)		
Race	White: 15 (88%)	African American: 2 (12%)			
Ethnicity	Non-Hispanic: 17 (100%)				
Performance Status	ECOG 0: 6 (35%)	ECOG 1: 9 (53%)	ECOG 2: 2 (12%)		

17 subjects who failed all standard therapies were enrolled in this 1st in human study with 9 evaluable for clinical response

- **4 received NEO-201 at dose level 1 (1 mg/kg)**
- **7 received NEO-201 at dose level 2 (2 mg/kg)**
- **6 received NEO-201 at dose level 1.5 (1.5 mg/kg)**

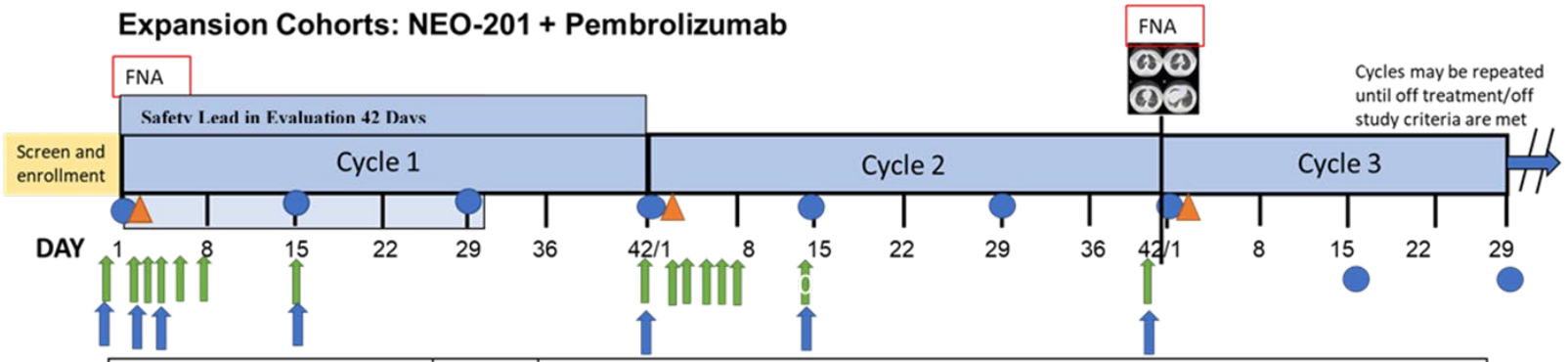
1.5 mg/kg was declared the maximum tolerated dose (MTD), and given the pharmacokinetic results was chosen as the recommended phase 2 dose

NEO-201 + Pembrolizumab Phase 2 Clinical Trial

Objectives

- Determine Objective Response Rate (ORR = CR, PR, SD) as determined by RECIST v1.1 guidelines and progression free survival (PFS) in four cohorts of subjects (NSCLC, HNSSC, uterine and cervical cancers) receiving NEO-201 at 1.5 mg/kg in combination pembrolizumab at the FDA approved adult dose
- Assess immunogenicity of NEO-201 in adults with relapsed or chemo-resistant solid tumors participating in the first 10 subjects receiving combination therapy.

Study Schema



STABILITY TESTING: NEO-201 DRUG PRODUCT

1) **PHOTOSTABILITY TESTING:** test to evaluate if the solution is clear, colorless to slightly yellow solution. Antibodies, especially their aromatic residues, are very sensitive to light. Light may induce photodegradation and fragmentation of protein structure

2) **SPECIFICATION**:

- **Ph** (range 6.0 to 7.0): Ph values outside this range can affect the affinity for the target antigen

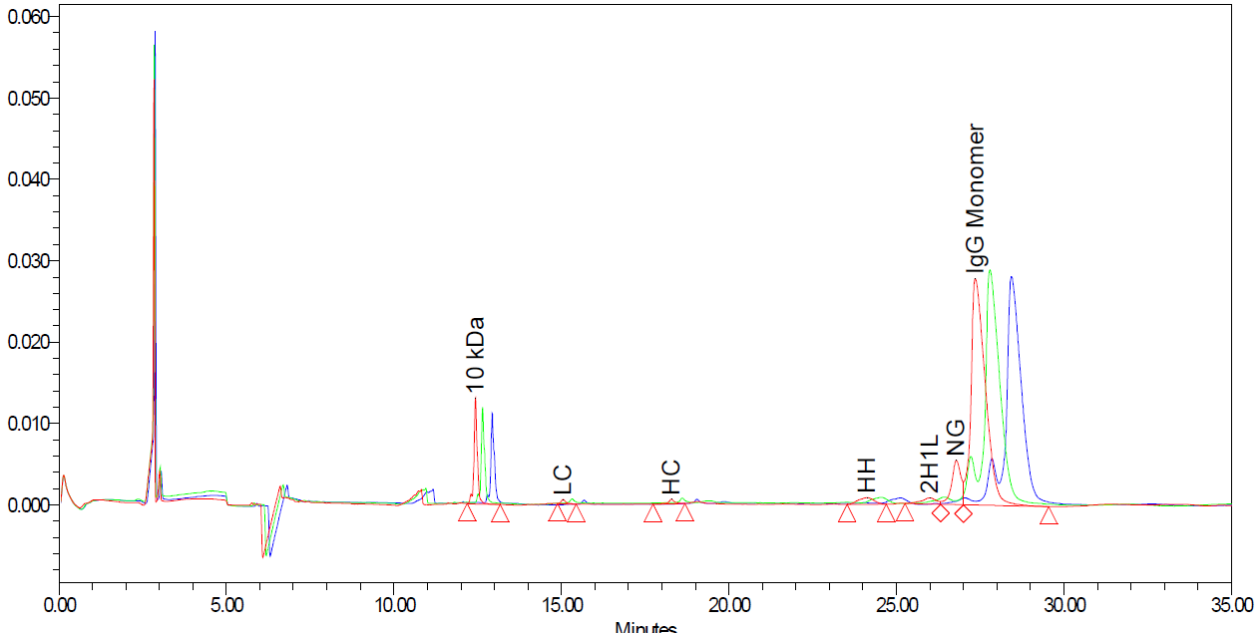
- **Osmolality** (range 360 ± 60 mmol/kg): Osmolality is important for clinical administration of the drug: final product must be isotonic for injection

- **Protein concentration** (range 9-11 mg/mL): NEO-201 is 10.2 mg/ml. Concentration lower than the range → PROTEIN DEGRADATION

STABILITY TESTING: NEO-201 DRUG PRODUCT

2) SPECIFICATION:

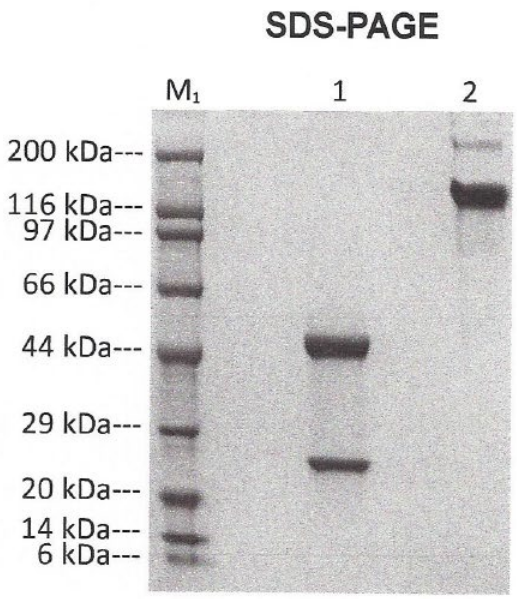
- **Size Exclusion HPLC:** evaluate the presence of aggregates or fragments. High protein concentrations seem to increase the viscosity of solutions, which itself may increase the aggregation potential of proteins. This concentration-dependent tendency to aggregation is an increasing concern in the administration of monoclonal antibodies which require highly concentrated solutions.

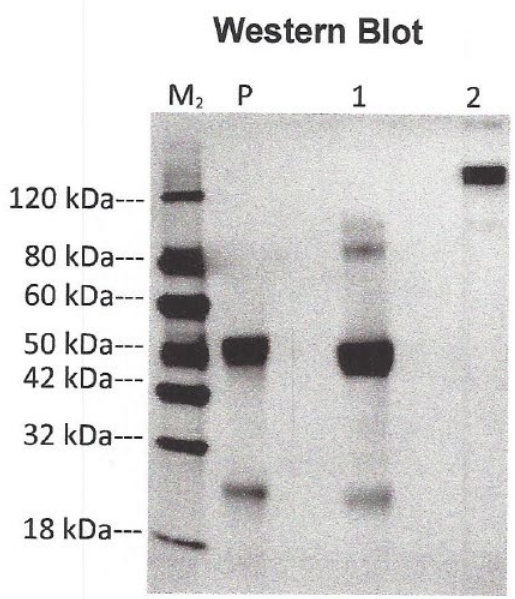


STABILITY TESTING: NEO-201 DRUG PRODUCT

2) SPECIFICATION:

- **CE-SDS in reducing and non reducing conditions:** ≥ 85% Heavy Chain + Light Chain and ≥ 90% Intact IgG structure : evaluate the integrity and structure of the antibody. A loss of its structure affects its binding affinity to the target antigen or its biological activity (ADCC, CDC).





STABILITY TESTING: NEO-201 DRUG PRODUCT

2) SPECIFICATION:

- **Particulate matter:** (range ≤6000 of ≥10 µm per container; ≤600 of ≥25 µm per container): Particulate matter contamination of pharmaceutical products can cause significant harm to patients. It is very important to check particulate matter contamination of injectable drug products before administration to patients.

- **Endotoxin assay**: ≤ 0.5 EU/mg : Endotoxin test is the most critical quality control test required by the FDA for all drugs in their final stages of formulation. Endotoxins are produced by gram-negative bacteria, and they are associated with severe reactions in humans. Toxins can retain high toxic activity even at low concentration. Endotoxins seem to be involved in the occurrence and development of many different diseases in humans.

STABILITY TESTING: NEO-201 DRUG PRODUCT

3) STORAGE CONDITIONS:

- **Temperature:** Low temperatures can induce protein denaturation, affecting both colloidal and conformational stability of proteins. Cold caused unfolding and aggregation is usually reversible, as the mAb mostly stays in a native conformation.
- Main temperatures tested for stability of NEO-201 are:

Study	Storage condition	Minimum time period covered by data at submission
Long term	5°C ± 3°C	12 months
Accelerated	25°C ± 2°C/60% RH ± 5% RH	6 months
Long term	- 20°C ± 5°C	12 months

STABILITY TESTING: NEO-201 DRUG PRODUCT

3) STORAGE CONDITIONS:

- **Upright vs Inverted sample:** Injectable drugs stored in glass bottles need to be tested in the **upright position** and in the **inverted position** to ensure contact of the solution with the rubber stopper of the container. Upright and Inverted samples are assayed to evaluate if the position affects:
- concentration
- pH
- Loss of potency
- Aggregate formation

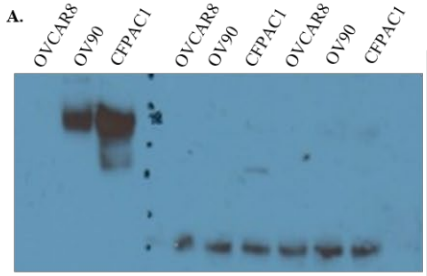
NEO-201 stability samples tested at different time point:

- 5°C upright
- 5°C inverted
- -20°C upright

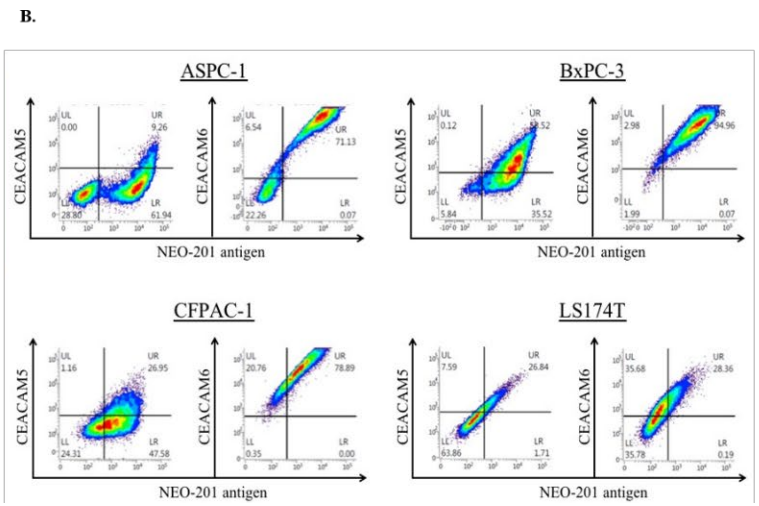
STABILITY TESTING: NEO-201 DRUG PRODUCT

STABILITY TESTS TO EVALUATE MAINTENANCE OF BIOLOGICAL ACTIVITY AS A MEASURE OF POTENCY:

- **ELISA: evaluate the stability of NEO-201 binding to its target antigen**



PEPTIDE	CFPAC1	OV90	#AAs	MW	Calc pi
CEACAM-6	4	3	344	37.2	5.82
CEACAM-5	1	2	702	76.7	5.69
CEACAM-7	1	2	265	29.4	5.54



CELL LINE (CANCER TYPE)	MARKER	% POSITIVE	MFI
ASPC-1 (pancreas)	NEO-201	**75.86**	**9,078**
	CEACAM5	20.04	869
	CEACAM6	**77.87**	**52,138**
BxPC-3 (pancreas)	NEO-201	**97.41**	**5,259**
	CEACAM5	**79.54**	**711**
	CEACAM6	**98.45**	**18,690**
CFPAC-1 (pancreas)	NEO-201	**85.21**	**1,728**
	CEACAM5	25.83	1,108
	CEACAM6	**96.50**	**27,792**
LS174T (colon)	NEO-201	29.15	858
	CEACAM5	36.34	1,030
	CEACAM6	**63.41**	**1,462**

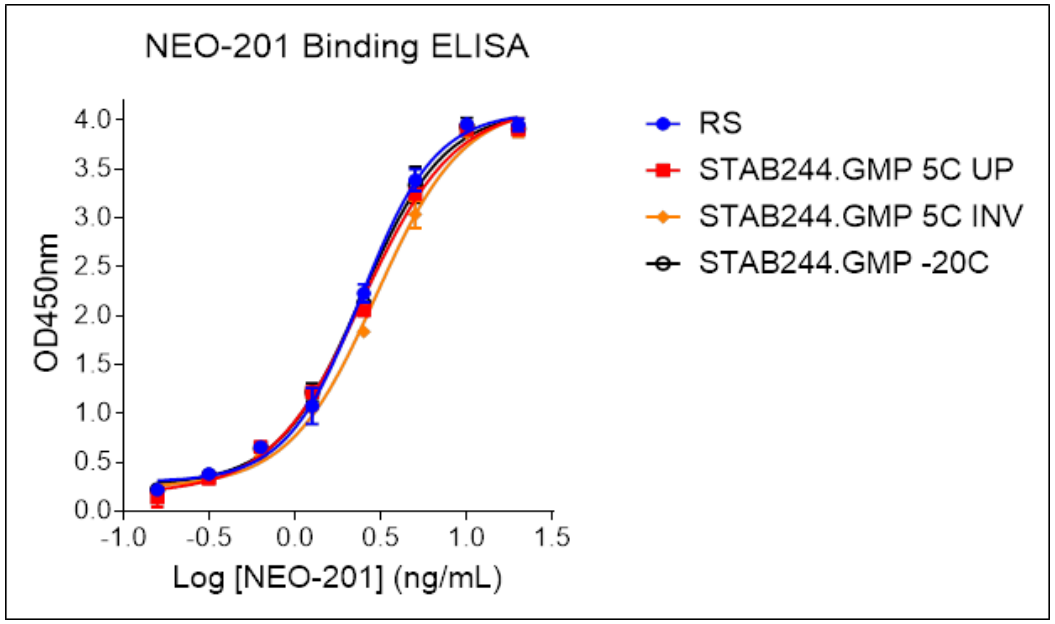
From these screening the Carcinoembryonic Antigen-Related Cell Adhesion Molecule (CEACAM)5, also known as CEA, and CEACAM6 were identified as the most likely targets of NEO-201

***Zeligs** et al. **Frontiers in Oncology**, 2020*

STABILITY TESTING: NEO-201 DRUG PRODUCT

STABILITY TESTS TO EVALUATE MAINTENANCE OF BIOLOGICAL ACTIVITY AS A MEASURE OF POTENCY:

- **ELISA: evaluate the stability of NEO-201 binding to its target antigen**

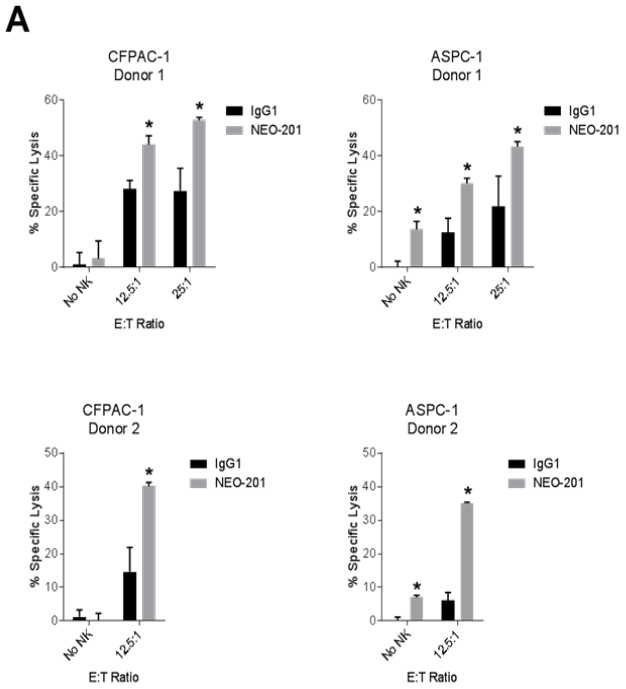


- Coat a 96 well plate overnight with Recombinant human CEACAM6 at 400 ng/mL
- Compare NEO-201 reference standard sample with NEO-201 drug product stability samples at different antibody concentrations

STABILITY TESTING: NEO-201 DRUG PRODUCT

STABILITY TESTS TO EVALUATE MAINTENANCE OF BIOLOGICAL ACTIVITY AS A MEASURE OF POTENCY:

- **ADCC assay: evaluate the stability of NEO-201 to maintain the capacity to mediate ADCC activity against human cancer cell lines expressing its target antigen**



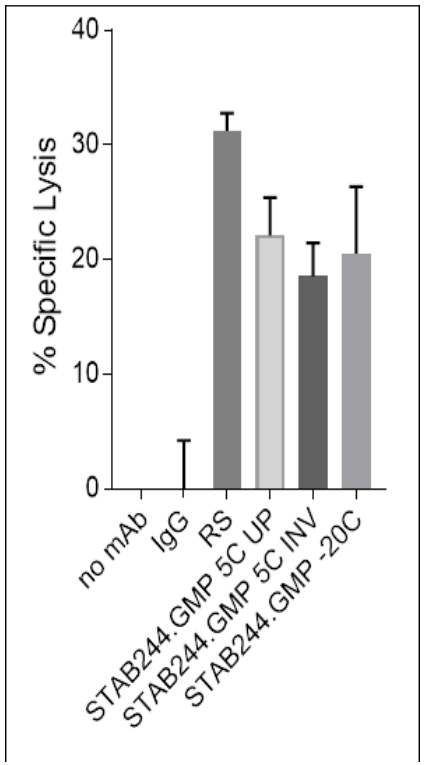
(**A**) ADCC activity using CFPAC-1 or ASPC-1 cells as target cells. Cells were treated with 10µg/mL of NEO-201 or human IgG1 (negative control). Purified NK cells from two healthy donors were used as effector cells at the indicated E:T ratios. (**B**) ADCC assay using CFPAC-1 cells treated with increasing doses of NEO-201. NK cells were used at an E:T ratio of 12.5:1.

Fantini *et al.* ***Frontiers in Immunology*, 2018**

STABILITY TESTING: NEO-201 DRUG PRODUCT

STABILITY TESTS TO EVALUATE MAINTENANCE OF BIOLOGICAL ACTIVITY AS A MEASURE OF POTENCY:

- **ADCC assay:** 4h non radioactive assay



Target cells: ASPC-1 (pancreatic cancer cell line expressing high percentage of NEO-201 target antigen)

↓

Target cells labeled with CALCEIN AM for a non-radioactive ADCC assay

↓

Target cells treated with **10µg/mL of NEO-201** or human IgG1 (negative control**). Purified human NK** cells are used as **effector cells at the 12.5:1 E:T ratio**.

STABILITY TESTING: NEO-201 DRUG PRODUCT

STABILITY TESTS TO EVALUATE MAINTENANCE OF BIOLOGICAL ACTIVITY AS A MEASURE OF POTENCY:

- **ELISA acceptance criteria:**

Controls	Acceptance Criteria	Result	Pass/Fail
No 1° Ab control	OD < 0.150		
No 2° Ab control	OD < 0.150		
Standard Curve of RS	$R^2 \geq 0.980$		
EC50 of RS	<10 ng/ml		
EC50 of Test Sample	± 250% of RS	1. 2. 3. 4.	

STABILITY TESTING: NEO-201 DRUG PRODUCT

STABILITY TESTS TO EVALUATE MAINTENANCE OF BIOLOGICAL ACTIVITY AS A MEASURE OF POTENCY:

- **ADCC assay acceptance criteria:**

Results		Acceptance Criteria		Pass/Fail
Spontaneous Lysis (% lysis)		<30%		
NEO-201 Reference Standard (RS, % lysis)		(RS-IgG)/IgG*100>50%		
NEO-201 Test Samples (% lysis)	1. 2. 3. 4.	Sample % lysis within ± 50% of the % lysis of reference standard (RS) as tested against human tumor cells at 12.5:1 effector/target cell ratio (Sample –RS)/RS ? ±50%	1. 2. 3. 4.	

CONCLUSIONS

PRECISION BIOLOGICS

1) Stability tests provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light and evaluate shelf life for the drug product and recommended storage conditions (example 5°C upright, 5ºC inverted, -20ºC, -60ºC)

2) Stability of monoclonal antibodies used in cancer immunotherapy is crucial to maintain their integrity and biological function: alteration of Ph, degradation, denaturation, contamination with toxin can impair monoclonal antibody safety and efficacy

3) Main tests to evaluate biological activity of a monoclonal antibody that mediate ADCC against human cancer cells:

- **ELISA** (evaluate stability of binding to the target antigen)
- **ADCC ASSAY** (evaluate the stability to maintain monoclonal antibody capacity to mediate ADCC)