

RP215-CAR-T

CAR-T Therapy for a Novel Target CA215 Expressed on Multiple Malignancies

Overview

Drug Name	RP215-CAR-T
Description	RP215-CAR-T is a chimeric antigen receptor (CAR) T cell technology developed a monoclonal antibody, RP215, which reacts specifically with cancer specific CA215 antigen. CA215 is a carbohydrate-associated epitope located on the heavy chains of immunoglobulins, which are expressed on the surface of almost all cancer cells including those from the ovary, cervix, lung and liver. In contrast to cancer expression, CA215 is absent on the immunoglobulins secreted by normal immune cells, making CA215 an ideal tumor specific antigen for CAR-T design. The monoclonal antibody against CA215 is designated as RP215, which has been engineered into the CAR construct with an improved design of co-expression of recombinant cytokine. RP215-CAR is able to target CA215 via scFv of humanized RP215, activate T cells via intracellular signaling domains, and regulate T cell response via cytokine element. In preclinical studies, RP215-CAR T cells specifically target cancer cells and induce strong apoptosis and cell lysis in these cells, indicating their therapeutic potential for treatment of a wide variety of cancers.
Target	CA215
Drug Modality	Chimeric Antigen Receptor-Modified T Cell
Indication	Cancer
Product Category	Immunotherapy
Mechanism of Action	RP215-CAR-T recognizes tumor cells expressing CA215 through the CAR of RP215, and then T cells are activated and exert immune effects to kill tumor cells.
Status	Pre-clinical
Patent	7 patents have been granted for RP215-CAR-T and RP215 monoclonal antibody worldwide.

Seeking Global Cooperation

Protheragen Inc. is interested in partnering with companies to further develop RP215-CAR-T pipeline, either in the form of a strategic alliance, licensing, or marketing agreement. We look forward to cooperating with you in the near future.

Target

CA215

Introduction

CA215, a cancer-associated antigen with minimal molecular weight of about 60 KDa, has not yet been developed for medical use. This antigen was initially identified by a monoclonal antibody designated as RP215. This monoclonal antibody was generated through immunizations of mice with cell extract of OC-3-VGH ovarian cancer cell line followed by cell fusions and screening of nearly 3,000 hybridomas. With RP215 as the probe, CA215 may be suitable for the monitoring of patients with various types of cancers including those of ovary, cervix, breast, lung, endometrium, stomach, colon and esophagus, but not with any of normal human tissues. The carbohydrate-associated epitopes of CA215 with pH-sensitive immunoactivity appear to be present only in cancer cell-derived immunoglobulins, but not in normal human immunoglobulins. Compared to normal immunoglobulin G, CA215 contains a significantly higher percentage of N-acetyl and N-glycoylneuraminic acid in the O-linked glycans, but a lower content of N-acetylglucosamine in the N-linked ones.

Molecular Size

50 to 70 KDa

Expression Form

Secreted and membrane-bound monomeric forms

LocationThe variable domain of the human immunoglobulin heavy chains

Clinical Resources

Major Conditions	Cancers
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Indication

Cancers Expressing CA215

Extensive IHC studies were performed with cancer cells of several different human tissues. As shown in the table below, cancer tissues of ovary, cervix, endometrium, stomach, esophagus and colon revealed greater than 50% of positive staining with RP215 monoclonal antibody. On the other hand, breast and lung cancer tissues exhibited only 30-35% of positive staining. Benign ovarian tumor specimens gave a significantly lower percentage of positive staining by ovary. These observations strongly suggest that CA215 can be expressed in large quantities on the surface of cancer cells and RP215-CAR-T is expected to be a potential immunotherapy for cancer.

Summarized results of immunohistochemical staining of cancer tissues in humans

Cancer tissues (Case No.)	Positive cases	Negative cases	Percentage positive (%)
Ovary (87)	56	31	64.4
Cervix (51)	43	8	84.3
Endometrium (36)	28	8	77.8
Stomach (93)	46	47	49.5
Colon (87)	38	49	43.6
Esophagus (56)	40	16	75.7
Lung (58)	18	40	31
Breast (59)	19	40	32.2
Liver (60)	2	58	3.5
Prostate (22)	2	20	9.1

Ovary (Benign) (31)	2	29	6.5
Cervix Cancer	<p>Cervical cancer, or malignancy of the uterine cervix, is one of the most important causes of cancer morbidity and mortality among women worldwide. The International Agency for Research on Cancer (IARC) determined that 569,847 incident cases of cervical cancer were diagnosed worldwide in 2018. Squamous cell carcinomas arising from the metaplastic squamous epithelium of the cervical transformation zone account for approximately 70% of all cases of cervical cancer. Cervical adenocarcinomas arising from the columnar epithelium of the endocervix account for around 25% of cervical cancer cases in countries such as the U.S., and appear to be increasing in frequency. Adenosquamous carcinoma, the least common form, accounts for 3-5% of all cases. Invasive cervical carcinoma requires aggressive and often multi-component therapy including surgery, external-beam radiation, brachytherapy and chemotherapy. Typically early cervical cancer is treated surgically, while concurrent chemoradiotherapy is the preferred modality for locally advanced cervical cancer.</p>		
Endometrial Cancer	<p>Endometrial cancer (EC) originates in the endometrium, the inner lining of the body of the uterus (corpus uteri), and is classified into type I or type II. EC is the fifth most common cancer in women worldwide, and continues to increase in both incidence (by 1-2% yearly) as well as prevalence. Although the prevalence of endometrial cancer is high, the mortality-to-incidence ratio is lower than that of other gynecological malignancies. Most patients do well with surgery alone. Five-year survival rates for women with FIGO stage I-II endometrial cancer range from 74% to 91%.</p>		
Esophageal Cancer	<p>Esophageal cancer refers to a malignant tumor of the esophagus which is the tube that connects the throat with the stomach. The two main sub-types of the disease are esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). Esophageal cancer is the eighth most frequently diagnosed cancer worldwide, and because of its poor prognosis it is the sixth most common cause of cancer-related death. In general, a combined approach that includes surgery may be considered as a curative intention of localized esophageal cancer. In the case of disease that is widespread and metastatic, chemotherapy may be used to lengthen survival, while treatments such as radiotherapy or stenting</p>		

may be used to relieve symptoms and make swallowing easier.

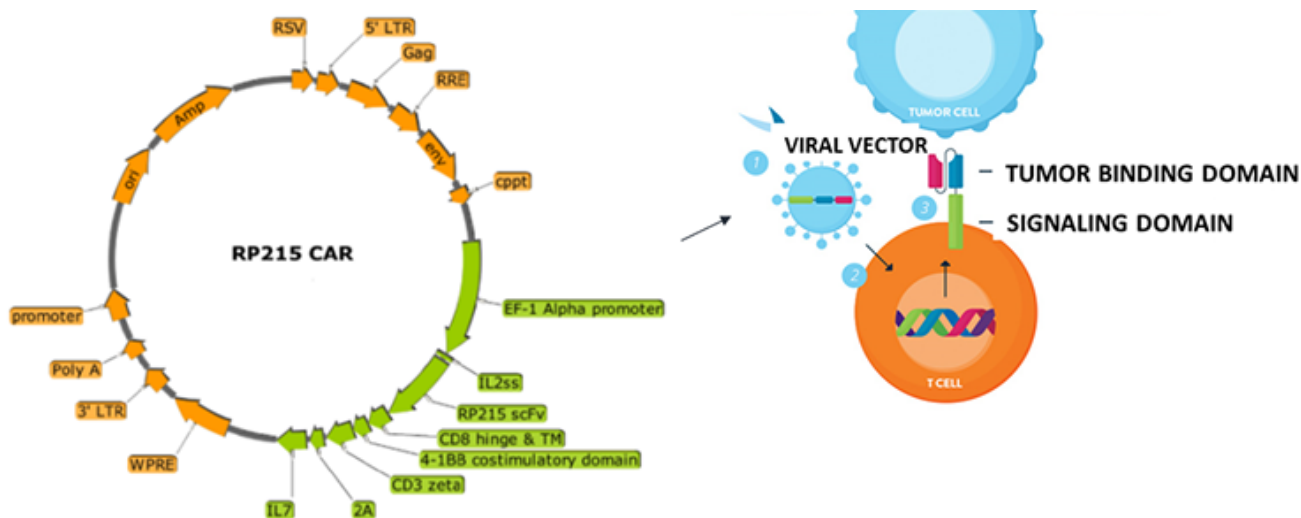
Ovarian Cancer

Ovarian cancer typically spreads via local shedding into the peritoneal cavity, followed by the implantation of high-grade papillary serous tumors on the peritoneum and local invasion of the bowel and bladder. The cancer spreading to pelvic lymph nodes is also common and it increases with the stages of disease. According to Globocan data for 2018, the global incidence of ovarian cancer would be 295,414. Worldwide, the five-year survival rate in average is 30-40%. At present, the two main ovarian cancer therapies are surgery and chemotherapy, alone or in combination depending upon the individual characteristics of the patient and tumor.

Mechanism of Action

RP215-CAR-T Cells Bind to CA215 to Be Activated and Kill Tumor Cells

RP215-CAR-T cells are autologous T lymphocytes that are genetically engineered to express binding site of RP215, thereby directing the autologous polyclonal T cells to bind CA215 expressed on tumor cells. The construct of CAR is composed of a single-chain variable fragment (scFv) of RP215 fused to the activating intracellular-signalling domain of the T-cell receptor (TCR) and 4-1BB domain. RP215-CAR-T cells recognize CA215 through the antibody domain, activating T-cell to proliferate and exert immune effects independent of major histocompatibility complex (MHC) presentation. Second-generation CAR designs are used for RP215-CAR-T, which incorporates one co-stimulatory signalling domain. Second-generation receptors are capable of delivering both a primary activation signal through the TCR ζ -chain and a co-stimulatory signal through the 4-1BB domain in the cytoplasmic tail. Clinical studies showed that second-generation CARs resulted in improving the in vivo expansion and persistence of the transfected T cells.



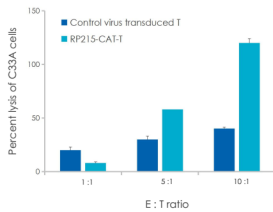
Status

The Patent Status of RP215-CAR-T

7 patents have been granted for RP215 monoclonal antibody, CAR construct of RP215, and methods of making and using worldwide.

	Discovery/Optimization	Pre-clinical	Phase I	Phase II	Phase III	NDA Filed
RP215-CAR-T	▶					

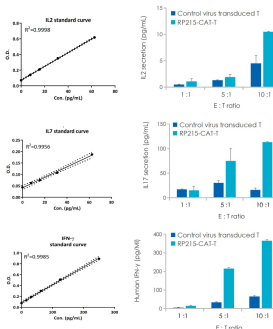
Data



In vitro Cytotoxicity of RP215-CAR-T

C33A cancer cells (T) were co-cultured with RP215-CAR-T cells (E) or with control virus transduced T cells at three different E/T (Effect/Target) ratios for 6 hours. The percentage of tumor cells lysis was measured by lactate dehydrogenase (LDH) levels.

In the E/T ratio of 5:1 and 10:1, RP215-CAR-T significantly increased the cell lysis compared with control T cells.



Cytokine Assays (ELISA)

After C33A cancer cells (T) co-cultured with RP215- CAR-T cells (E) or with control T cells, the release levels of three cytokines involved in the immune response were measured by ELISA.

IL2, IL7 and IFN- γ play important roles in the immune response involving T cells. As shown in the figures, RP215- CAR-T cells significantly increased the release levels of three cytokines compared with T control cells, especially in the E/T ratio of 5:1 to 10:1. Dose-dependent increases in the levels of three cytokines suggested that RP215-CAR-T cells could kill C33A cells through a series of immune responses.