# VAXO-Q-RE Therapy

Comprehensive Guide

## What is VAXO-Q-RE Therapy?

- VAXO-Q-RE is best described as an Autologous Adoptive Cellular Therapy. It consists of the basic players of innate and adaptive immunity, i.e., macrophages and Natural Killer cells (innate immune cells) and activated Dendritic Cells, T cells and antibody producing plasma cells (adaptive immune cells). Innate immune cells are ready to fight any pathogen or cancer cell in an antigen independent manner, while T cells and B cells require pre- activation with antigenic peptides. Activation of adaptive immune cells is performed with dendritic cells, a type of antigen presenting cells (APC). All these cells can be generated from a patient's blood and then be infused back to the patient.

## How does VAXO-Q-RE work?

- Action role of immune cells: Macrophages and Natural Killer cells can recognize and kill foreign pathogens and cancer cells. Macrophages are phagocytic cells and Natural Killer cells both, can eliminate cancer cells in an MHC-independent manner. Cytotoxic T-cells (CTLs) are activated against specific antigens by dendritic cells and have the ability to target and kill cancer cells expressing the specific antigen. Antibodies produced by plasma cells, tag cancer cells and help their recognition by phagocytes or complement proteins leading to their subsequent destruction.
- RGCC's laboratories uses a patient's isolated peripheral blood mononuclear cells as a source for the in vitro production of VAXO-Q-RE. Macrophages and Natural Killer cells are selected and expanded in large numbers. Monocytes are used as a source for the production of dendritic cells (DCs). DCs are then pulsed with synthesized peptides designed specifically to mount an immune response against tumor proteins. These peptides are selected carefully so as to activate both T-lymphocytes (CTLs) as well as antibody producing cells aiming at long term memory cells and overall protection. VAXO-Q-RE is composed of three doses, containing innate immune cells (macrophages and Natural Killer cells) and adaptive immune cells (CTLs and antibody producing cells).

## What is the goal of VAXO-Q-RE?

- Boost Natural Immune System defence against the patient's cancer
- Activation of immune cells with patient's tumor antigens

**What kind of cancers can be treated with VAXO-Q-RE?**

- Theoretically all blood and solid tumor cancers can be treated if a circulating tumor cell can be found in the blood. Patient needs to be staged with at least a Stage 2B cancer.

**What kind of cancers will not respond to VAXO-Q-RE?**

- CNS cancers due to the blood brain barrier.

**At what cancer stage should a patient consider VAXO-Q-RE?**

- A patient must be staged at a minimum of grade 2B
- A patient can consider VAXO-Q-RE when all other options have been exhausted
- If the disease is stable and the patient is not on any chemotherapy or radiation therapy concurrently.

**Are there any contraindications to VAXO-Q-RE?**

- Recent radiation or chemotherapy - the patient will need to wait a minimum of three (3) weeks.
  - The time frame is only an estimate and may be longer depending on the status of the patient, and their immune system's ability to restore itself. Careful evaluation needs to be done by the healthcare provider before recommending this therapy to insure optimal response.
- Recent blood transfusions – the patient will need to wait 120 days
- Active Autoimmune Disease
- Cachexia
- Pregnancy or breast feeding
- Active infections/inflammation (CRP>3.0) (Sed Rate >29 mm)
- High Tregs or TNF-a on the pre-Immune Frame. (Over 5%)
- Children under the age of 18.

**Why can't VAXO-Q-RE be done on children?**

- VAXO-Q-RE has not been evaluated in children. They have an immature immune system and we do not know the effect the immune system and development of self-tolerance. Additionally, children have an active thymus gland and their immune system is under development. Therefore, we do not recommend it.

**Is VAXO-Q-RE safe?**

- VAXO-Q-RE is an Autologous Adoptive Cellular Therapy and is generally a very safe procedure. Adverse events are generally mild and include: flu-like symptoms, fever, injection site reaction (skin rash), pain, swelling and inflammation at tumor site are all possible reactions.

**What happens if the patient's immune system doesn't respond?**

- This therapy is creating the required immune response ex vivo, so there will be a response. The particular level of response will be dependent on the status and health of the patient as well as if the patient adhered to the post therapy guidelines.

**What is the administration and blood draw timeline for the VAXO-Q-RE?**

- Initial blood draw for therapy on day -15
- Therapy infusions are on days 0, 15 and 30
- Follow up Immune Frame and Oncotrace are drawn on day 120*
- Follow up Immune frame and Oncotrace are drawn on days 210*

**Note**: If the administration schedule was altered at all, then the follow up tests at day 120* are calculated at 90 days after Dose three (3) and then another 90days after that for day 210* follow up tests.

**Important:** These are live cells and need to be kept cold. Watch tracking closely to ensure the therapy will be arriving during office hours. Put into the refrigerator upon receipt. Do not freeze. The dendritic cells must be administered by Day 7 at latest (Shipment from lab is on a Friday (Day 1 ) administration must occur by Thursday of the following week (Day 7). After Day 7 the cells are no longer viable and cannot be administered.

**Is the dosing timeline flexible for VAXO-Q-RE?**

- It is recommended to follow the recommended administration schedule to ensure that the patient receives the highest viability/effect.
- Both doctors and patients need to schedule the administration of all three doses in advance to receiving dose one (1) in order to avoid any delays with the additional two doses that will be upcoming.
- Should there be an unexpected delay, the therapy can be requested to be shipped and administered up to one week late without loss of efficacy. Past this window, the therapy loses its potency and the patient will not receive the full benefit.

**What is <u>included</u> in the VAXO-Q-RE package and what is the cost?**

- Package price: 7000 Euro
- Package Includes:
    - All three doses of the VAXO-Q-RE Therapy
    - 2 Follow up Immune Frames – Day 120 and Day 210
    - 2 Follow up Oncotrace Tests – Day 120 and Day 210

- Package DOES NOT include:
    - Initial (baseline) Immune Frame (800 Euro) & Oncotrace (650 Euro)
    - Additional follow up Immune Frames (past the Day 120 and Day 210 one) (800 Euro)

**What are the <u>mandatory pre-requisite tests</u> to establish a baseline and eligibility for VAXO-Q-RE?**

- Immune Frame (Initial)
- Draw: 15-25 ml of blood
- Cost: 800 Euro

- Oncotrace (Baseline CTC)
- Draw: 15 ml of blood
- Cost: 650 Euro

(**Reminder**: the <u>baseline tests are not included in the cost</u> of the actual therapy)

**What is the mandatory follow up tests for VAXO-Q-RE?**

- Mandatory Follow up Testing: **Day 120**
- **Immune Frame** for verification of immune response (draw 15-25ml blood)
- **Oncotrace** for the follow up cell (CTC) count (draw 15ml of blood)

- Mandatory Follow up Testing: **Day 210**
- **Immune Frame** for verification of immune response (draw 15-25ml of blood
- **Oncotrace** for the follow up cell (CTC) count (draw 15ml of blood)

**Note**: Lab will evaluate these results and if patient is not at expected levels, a second round of VAXO-Q-RE may be recommended.

**How much blood is required for VAXO-Q-RE?**

- Initial Immune Frame (Baseline test) (Draw 15-25ml of blood)
- Oncotrace (initial CTC – baseline test) (Draw 15ml of blood)
- Dose 1, 2 and 3 of VAXO-Q-RE (Draw **120-140ml** of blood)
- Follow up Immune Frame (Draw 15-25 ml of blood)
- Follow up Oncotrace (Draw 15ml of blood)

**NOTE:** if ordering the Oncotrace, Immune Frame and all the three doses of the therapy at the same time then **150ml of blood (5 vials)** will be sufficient.

It is **highly recommended** to evaluate the baseline Oncotrace and the initial Immune Frame **prior** to ordering the therapy to verify the patient's eligibility. **30ml of blood (1 vial)** is sufficient for both tests if ordered at the same time.

**What pre-medications are required for VAXO-Q-RE?**

PRE-MEDS: Administer pre-medications prior to each dose of the THERAPY.

- **Recommended**: 4 mg dexamethasone I.V. in a 20-50 ml rapid drip saline solution or slow bolus push.
- Optional: IV H2 inhibitors: Cimetidine, Nizatidine, or Famotidine-give at least 30 min before IV.

- Optional: Paracetamol (Acetaminophen), PO 500mg, three time per day for up to three days starting an hour before the application, in order to counteract headache that could develop.

**What needs to be avoided <u>prior</u> to the Pre-Immune Frame and the VAXO-Q-RE?**

- **<u>Pre-Therapy Administration</u>:** The patient must be off ALL cytotoxic and free radical producing therapies. If drawing for cellular therapies, the patient must be off ALL immune suppressing therapies as well.
  - **Natural Substances** (IV): cytotoxic substances like Vitamin C or Ozone at least **14 days**
  - **Natural Substances** (oral supplements): Class 1 cytotoxic substances (per patient's Onconomics Plus results) at least **14 days.**
  - **Chemotherapy** (non-platinum derivative): at least **14 days**
  - **Chemotherapy** (platinum derivative): at least **21 days**
  - **MOAB or SMW drugs** for at least **14 days**
  - **Blood Transfusions:** at least **120 days**
  - **Radiation**: at least **14 days**
  - **Contrast:** at least **14 days**
  - **Surgery** (simple/routine): at least **7-10 days**
  - **Surgery** (brain or extensive): minimum of **30 days** based on time of recovery. Could be longer if slow recovery or if the person had some type of adverse reaction. Must be evaluated on a case-by-case basis.
  - **Fever:** at least **14 days**
  - **Hyperthermia** (local/concentrated/microwave ablation): at least **30 days** due to increase in cellular debris released into blood stream.
  - **Hyperthermia** (generalized/systemic): no waiting
  - **Cryoablation:** no waiting

- **Immune Suppression Medication** (All pre-Cellular Therapies – VAXO-Q-RE, Vaccine Prep, Dendritic Cells, DendroCov): at least **14 days.**
- **Radioactive Seeds:** Patients are not eligible for therapies due to the prolonged and undetermined time of the radiation exposure.
- **Gamma Delta T Cell Therapy (GDTC):** Patients are not eligible for therapies due to the potential interaction with RGCC therapies.

The purpose of these guidelines is to ensure the highest level of effectiveness of each therapy by removing treatments that interfere with and/or diminish the effectiveness of that therapy. Adherence to these guidelines will improve therapy effectiveness and patient outcomes.

**REASON:** The breakdown of the CTC caused by these substances creates debris that interferes with the therapy's ability to find its target. Allowing time for the body to clear the debris will increase the effectiveness of the therapy

**What needs to be avoided after VAXO-Q-RE?**

- The patient must stay off **ALL cytotoxic, or free radical producing, and immune suppressing therapies 120 days** after the administration of the therapy.

**The above is not an exhaustive list of problematic substances, so how can you decide what might interfere with the development of memory cells?**

In deciding what might or might not interfere with the development of the memory cells, ask yourself if the product has a direct or indirect effect on the CTC (in either being directly cytotoxic or in the generation of free radicals). Those are the problem substances since they create inflammation and debris in the blood sample (the scientists call it noise). Example: Artemisinin breaks down DNA so it' works directly as a cytotoxin so it must be avoided. So does substances like Ivermectin, Ozone, Colloidal Silver, and Curcumin.

However, substances that work indirectly through the metabolism of cells (starving cancer) like Salicinium or Metformin only need to be avoided for 7 days after the administration of the therapy.

Additionally, substances like Flavonoids (ALL – including Quercetin and Resveratrol) and products like modified citrus pectin also work indirectly so they also only need to be avoided for the 7 days after administration of the therapy.

**Are there any possible adverse reactions with VAXO-Q-RE?**

- Flu-like symptoms
- Fatigue
- Fever
- Injection site reaction (skin rash)
- *Uticaria (hives)
- Tumor Lysis Syndrome (TLS) with large volume of tumors
- Pain, swelling and inflammation at tumor site

*__Note__: There has never been any reported severe life-threatening anaphylactic reaction from this procedure. However, there is no guarantee that this will not happen, however rare it may be.

**What is the expected outcome with VAXO-Q-RE?**

Overall, preliminary results based on statistical analysis of CTCs and immune status, as well as on clinical evaluation and Karnofsky Index, indicate that there is an up-regulation of immunity. According to the ELISA analysis the activation of the humoral immunity based on overproduction of relevant factors like CD80 and CD86.

**What are the expected values on the Immune Frame after completion of the VAXO-Q-RE?**

Sample Day 120 – Expected results:

- T&B cells should be steadily present
- CD 80/CD 86 should be over 22%
- CD 28 – B & T line should be above 0 (even barely is ok)
- If memory cells are not well established then a second round of VAXO-Q-RE should be administered

Sample Day 210 (long term immunity is established) – Expected results:

- T&B cells (central) should be steadily present and
- CD 80 and CD 86 should be over 22%
- CD 28 on B and T lineage should be above 1%

**Is there any data on outcomes with the VAXO-Q-RE?**

The newest article that has been recently published regarding the VAXO-Q-RE therapy outcome can be viewed here: https://www.sciencedirect.com/science/article/pii/S0008874922001411

**How is it determined if the patient needs a Booster?**

Whether the patient will need a booster or a repeat of the VAXO-Q-RE will be advised by the Scientists at the lab. Please be sure that you have done your follow up requirements in terms of testing and data.

Send an email to **support@rgcc-international-northamerica.com** with patient's full name and why you are requesting a booster. The request will be submitted and once the lab has advised on their decision the doctor will be advised accordingly.

## How to Administer VAXO-Q-RE

**Important**: It is critical that doses be administered on the recommended intervals to ensure optimum response. There is a synchronization effect needed and it is essential the doses be added at the designated time, or the therapy will not work as intended.

**First steps**:

- Take the patient's VAXO-Q-RE therapy vial out of the refrigerator and allow it to come up to ambient room temperature while the pre meds are being given. Even warming to low normal body temperature (90-95 degrees) by holding in your hands can be very helpful as well.
- Check the vial number and match to patient's name.
- Inspect the vial. The cells are suspended in solution and are ready for administration. The solution should be colorless without any sign of precipitation. **In case of any color changes or any precipitation DO NOT ADMINISTER. Immediately notify RGCC by email at **info@rgcc-international-northamerica.com** and call 1-800-813-1372.

**PRE-MEDS: Administer pre-medications prior to each dose of the DENDRITIC CELL THERAPY.**

- Start with a 250 – 500 ml bag of saline
- Start the IV line with Catheter to the patient
- You can give the IV form of any H2 inhibitors: Cimetidine, Nizatidine, or Famotidine-give at least 45-60 minutes before the IV. If giving the medications orally, it needs to be given 6 hours before the IV.
- Ready 4 mg dexamethasone I.V. in a 20-50 ml rapid drip saline solution or very slow bolus push
- Paracetamol (Acetaminophen), PO 500mg starting an hour before administration and up to three times per day for up to three days after administration to help counteract the headache that could develop.

**Note**: The use of Dexamethasone is to help prevent the likely severe damage to the surrounding tissue if the patient experienced an extravasation during the administration of the therapy.

**Day 0 – 1st Dose (Macrophages and NK cells)**

- Before the pre-medications are finished prepare 250-500 ml IV saline bag
- Remove the security tape carefully, remove cap, wipe the stopper with a sterile alcohol swab.
- Leave the bottle in an upright position to remove the sample, REASON: (the inside rims on rubber seal can sometimes cause cells to stick)
- Use a 10 ml syringe with a 21-gauge 2-inch needle and very slowly remove the 6ml of cells
- Administer the cells via a slow IV push. Remember to be slow and gentle
- Immediately following the IV push start the 250-500 ml saline bag over a 30-60 minute rapid drip
- This drip should not cause any pain or discomfort to the patient. In some cases, during the IV or shortly thereafter, the patient may begin to experience a slight fever (99-100), slight headache, or chills. This is generally a good thing but monitor the patient while in your office and instruct them to call you if the fever rises over 102 degrees over the next several days.

**Day 15 – 2nd Dose** (APCs, CTLs and plasma cells) To be given 2 weeks after Dose 1

Administer exactly the same as dose 1

**Day 30 – 3rd Dose** (APCs, CTLs and plasma cells or Macrophages and NK cells or a mixture of what the patient needs as determined by RGCC) To be given 2 weeks after Dose 2

Administer exactly the same as dose 1 and 2

**Day 120 – <u>Mandatory</u> Follow up Immune Frame and Oncotrace**

Mandatory - Included in package

**Day 210 – <u>Mandatory</u> Follow up Immune Frame and Oncotrace**

Mandatory - Included in package

## Advanced FAQ from Practitioners

From Dr. Papasotiriou: The below questions are mainly based on knowledge of immune surveillance evasion in normal immune cells. In VAXO-Q-RE all processes have already taken place ex vivo and there is no dependency from stimulus or T4 cells.

**Q: Is Tumor hypoxia expected?**

A: Actually, hypoxia through HIF1a induces stemness and this is exactly the main targeted cells from the primed cells that composed VAX-O-QRE. So actually, hypoxia and is considered a favourable feature.

**Q: Recruitment of immune suppressive T-regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs)**

A: Tregs are generated from excess of antigens, and they require the presence of T4 helper cells. In VAXO-Q-RE all stimulation has already taken place ex vivo and the Tregs will not give any switch off signal. To be precise CTLs are not affected by Tregs as long as they become CTLs from T8. M1 and NK are not interacting with Tregs since they belong to the non-specific immunity system.

**Q: Recruitment of tumor-killing M1 macrophages and polarizing them into tumor-promoting M2 macrophages (also known as tumor-associated macrophages or TAMs)**

A: This would involve a change in the polarity from M1 to M2. For this to happen it would require the release of massive tumor antigens in the area and this is not going to happen due to the presence of APCs that regulates the immune response rate.

**Q: Impaired function of DCs by the intracellular accumulation of cholesterol.**

A: The DCs are generated and primed ex vivo, the formation of intracellular vesicles with cholesterol and lipids happens only when the DCs are generated in vivo.

**Q: Prostaglandin E2 (PGE2)-triggered immune evasion**.

A: Yes, but with a mechanism that is affecting the platelets and they form the micro emboli, this also requires the generation of primary niche which then the cancer cells need to interact with normal cells getting out of the micro emboli status. This moment is critical since they are exposed to immune surveillance and the only way to evade is not to present any antigen (which is impossible since they need them in order to interact with the stroma cells).

**Q: Iron-induced para fibrin formation blocking immune-mediated destruction.**

A: Again, you are referring to the formation of micro emboli but in this case, you need the presence of tPA to trigger this mechanism and it is not sufficient by itself. This is the main reason the PLTs are involved as well. Hence, we are falling to the same mechanism as before.

**Q: Tumor collagen density preventing adequate infiltration of immune cells.**

A: If you have ever touched a tumor, you know that the formation is very thin and very unsolid. The collagen that you are referring to is impaired and by far it is not solid enough to avoid immune cell's ability to penetrate. The biggest proof of this concept is the TIL (tumor infiltrated lymphocytes) which are present in all solid tumors.

**Q: Accumulation of activin A. Which activin you believe it is the most relevant A, B, AB, C?**

A: If you believe that Activin AA is the only thing tumors can form as dimmer, then think again. Each dimmer has a complete controversial mechanism. So, I would not always be confident that we have to consider only Activin AA in the cancer development case.

**Q: Lack of sufficient energy by immune cells.**

A: The cells in VAX-0-QRE are already triggered ex vivo and they are vigorous due to the fact that they are living in an ideal environment. So, in the period of their life span (which may be from 5 to 12 days) they have sufficient independency and are able to perform specific and nonspecific immune reactions.

**Further discussion:** Altered tumor energy metabolism induces an acidic extracellular micro-environment and is able to dampen the expression of major histocompatibility complex-1 (MHC-1), resulting in reduced expression of tumor associated antigens (TAAs).

And the final evidence is if all these reasons are making the immune system unable to fight the cancer cells, then why would a more simplistic approach of blocking of PD1 or PDL1 (which by the way is highly dependent on the body's stimulus and signalling) give us such promising results on immunotherapy? Also, why is all the therapy approached with APCs, NKs etc. showing such good results in actual cases.

So, my advice is that biochemical models are good, but they cannot explain more complex entities and since malignancies have way more complex interactions, we cannot rely only on the more simplistic model mechanism.