THERAPEUTIC VACCINATION WITH *EX VIVO*-GENERATED AUTOLOGOUS MONOCYTE-DERIVED DENDRITIC CELLS

- DCs were generated *ex vivo* from autologous monocytes, isolated from 300 ml of the patient's peripheral blood.
- Monocyte differentiation into immature DCs was induced using GM-CSF and interferon-α.
- Immature DCs were loaded with allogeneic tumor lysates (anaplastic astrocytoma + glioblastoma + melanoma) serving as a source of tumor-associated antigens (TAAs).
- Lysate-loaded immature DCs were matured using lipopolysaccharide and interferon-γ.
- Clinical-Grade Product Quality Control Certificate was issued based on morphological, immunophenotypical and functional evaluation of the generated DC vaccines.
- All stages of DC vaccine production were performed under Good Manufacturing Practice (GMP)-controlled conditions in an accredited laboratory (UAB "Froceth", Linkmenų g. 28, Vilnius LT-08217, Lithuania; Phone No: 00370 673 89527, E-mail address: info@froceth.lt).
- **Date of blood collection for the production of DC vaccines:** October 4, 2016.
- **Number of DC vaccine doses:** 9 doses, containing $5x10^6$ DCs per dose (8 doses) and $2.5x10^6$ DCs per dose (the first dose).
- All DC vaccine doses are stored in liquid nitrogen (–196° C) and are gradually thawed before each injection.
- Immediately before injection, each dose of DC vaccine is suspended in 1 ml of injection solution consisting of 0.9 % NaCl solution and autologous serum at a ratio of 1:1.
- Each dose of DC vaccine is injected intradermally and subcutaneously (50:50) in the external region of the upper arm.
- **Immune response to the treatment** is assessed by evaluating peripheral blood immune profile before starting the treatment and assessing its dynamics in the course of treatment as detailed below.

TREATMENT PLAN

Chemotherapy

Temozolomide (October 10–14, 2016) (I)

Chemoimmunotherapy

Temozolomide (November 7–11, 2016) (II)

November 15, 2016 1st DC vaccine (***right arm***) + ALDARA® 5% for 2 days
Adverse effects:

November 22, 2016 2nd DC vaccine (***left arm***) + ALDARA® 5% for 2 days
Adverse effects:

Temozolomide (December 5–9, 2016) (III)

December 20, 2016 3rd DC vaccine (***right arm***) + ALDARA® 5% for 2 days
Adverse effects:

Temozolomide (January 2–6, 2017) (IV)

January 17, 2017 4th DC vaccine (***left arm***) + ALDARA® 5% for 2 days
Adverse effects:

Temozolomide (January 30 – February 3, 2017) (V)

February 15, 2017 5th DC vaccine (***right arm***) + ALDARA® 5% for 2 days
Adverse effects:

Temozolomide (February 27 – March 3, 2017) (VI)

March 27, 2017 6th DC vaccine (***left arm***) + ALDARA® 5% for 2 days
Adverse effects:

April 24, 2017 **IMMUNOMONITORING**

Immunotherapy

May 8, 2017 7th DC vaccine (***right arm***) + ALDARA® 5% for 2 days
Adverse effects:

July 3, 2017 8th DC vaccine (***left arm***) + ALDARA® 5% for 2 days
Adverse effects:

September 4, 2017 **IMMUNOMONITORING**

The last (9th dose) will be injected after one year (in July, 2018) or in the case of disease recurrence.

ALDARA® (imiquimod) 5% cream is applied topically at the DC vaccine injection site (20 cm^2 area):

1. Immediately after DC vaccine injection;
2. In the morning of the next day after DC vaccine injection (wash the injection site gently in the evening).

Interferon alpha is administered only if blood parameters are favorable **(WBC > 3 × 10^9/L, NEU > 1.5 × 10^9/L, PLT > 100 × 10^9/L, RBC > 4 × 10^{12}/L, hemoglobin > 110 g/L)**:

1. Immediately after DC vaccine injection;
2. On the 2nd or 3rd day after DC vaccine injection.

Possible adverse effects associated with dendritic cell vaccination:

- Local reaction: redness, swelling, induration, tingling, pain of the injection site. These signs resolve within 1–5 days. Skin hyperpigmentation at the injection site can persist for a longer period of time (up to a several months).
- Enlargement and rarely pain of regional lymph nodes (axillary or inguinal). It resolves within 4–7 days.
- Cold (flu) symptoms, including subfebrile fever (< 38° C), chills, headache, rarely – discomfort or pain of joints or muscles. These adverse effects are controlled by administering 600 mg of ibuprofen (up to 2 400 mg per day) or 1 g of paracetamol (up to 4 g per day).
- Weakness, fatigue, sleepiness, malaise.
- ***Adverse effects are more severe, if low-dose interferon alpha is used with DC vaccination (the patient may experience moderate to severe flu symptoms lasting for up to 3 days), including:*** febrile fever (38–40° C), chills, headache, vertigo, joint and/or muscle pain, abdominal pain, nausea, vomiting, diarrhea, shortness of breath (dyspnoea), increased heart rate (tachycardia), malaise, weakness. These adverse effects are controlled by administering 600 mg of ibuprofen (up to 2 400 mg per day) or 1 g of paracetamol (up to 4 g per day).
- Allergic reactions are very rare (< 0.1% of cases). They are generally easily controlled by administering H_1 receptor blockers (e.g. loratadine – 10 mg 1x day, bilastine – 20 mg 1x day, etc.). The patient must be warned that he/she must call ambulance (112) immediately if severe allergic reaction occurs (no such cases have been reported since the start of DC vaccination in 1994). *Signs of severe allergic (anaphylactic) reaction:* localized and/or spreading redness, rash, swelling of skin and/or mucosa, progressive shortness of breath, increasing heart rate, low arterial blood pressure, nausea, vomiting, abdominal cramps, impairment or loss of consciousness, seizures, involuntary defecation and/or urination. Severe anaphylactic reaction is treated with systemic adrenomimetics, antihistamines, glucocorticoids, infusion therapy.
- No fatal adverse effects of therapeutic dendritic cell vaccination have been reported since 1994.

Marius M. Strioga MD/PhD
Oncoimmunologist
Department of Immunology
National Cancer Institute
P. Baublio g. 3b-321, LT-08406, Vilnius

Contacts:
Cell phone: 00370 601 69551
Office phone No: 00370 5 2190 932
strioga@gmail.com (personal e-mail)
marius.strioga@nvi.lt (office e-mail)