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The use of the drug Refnot® (α-tumor necrosis factor-thymosin-α1) in patients with disseminated skin melanoma

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Tumor Necrosis Factor

- **TNF-α (cachexin), TNF-β (lymphotoxin)**
- Production: stimulated macrophages and activated T-lymphocytes
- Binds to TNF-R1 and TNF-R2 receptors
- From 500 to 10,000 high-affinity receptor to TNF are present at all somatic cells, except erythrocytes
- Mediator of inflammation, activator of the cellular immune response and apoptosis in cancer cells

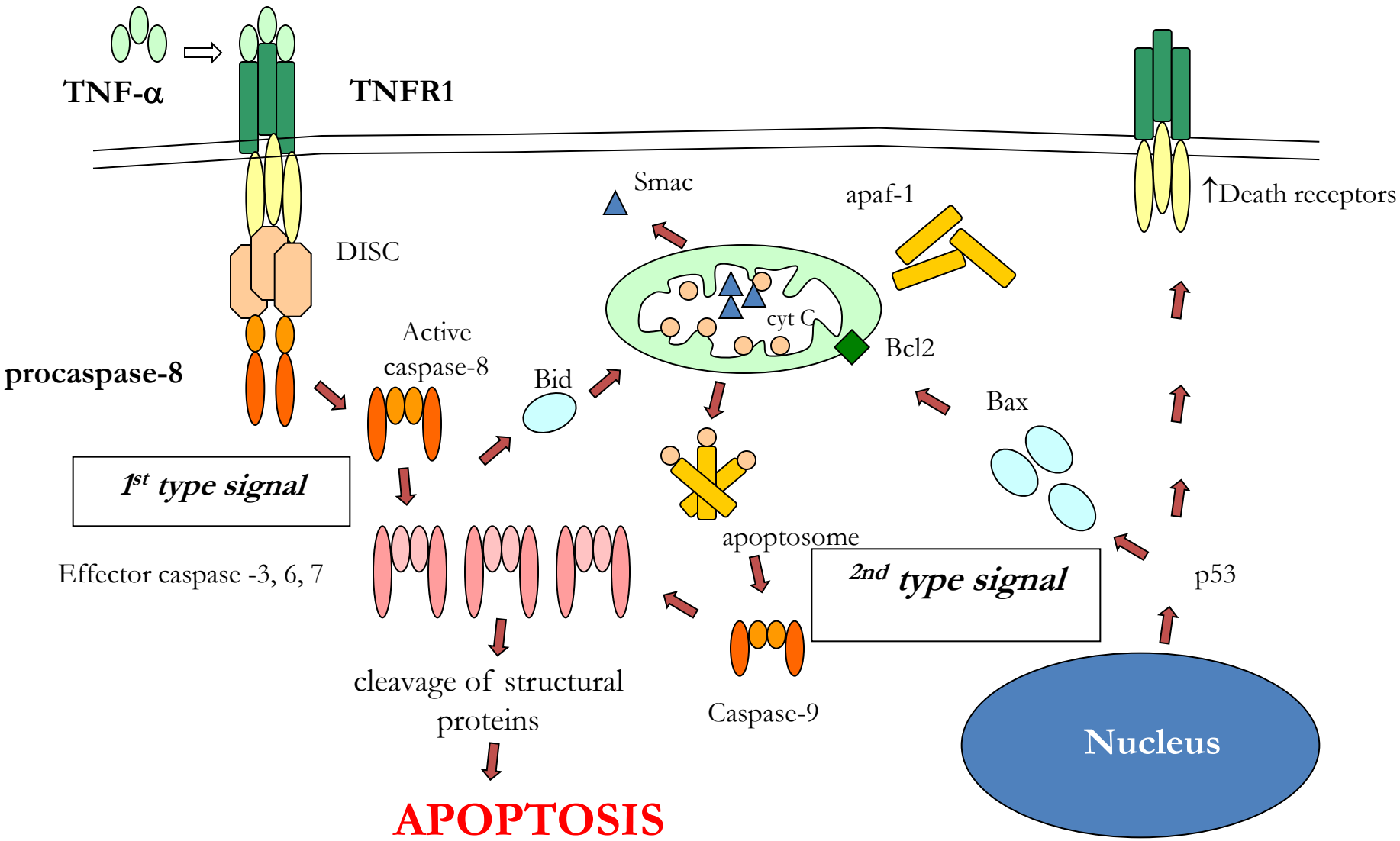
Tumor necrosis factor - alpha

- **Tumor necrosis factor – alpha (TNF-α)** was selected in the experiment in 1975
- Since 1984 the began the first clinical study of recombinant TNF-a
- The high toxicity to humans was marked in systemic administration
- It was the limitating factor, that is why the desired therapeutic effect was not achieved

Mechanisms of antitumor action of TNF-α

- The impact on the receptors of tumor cells with the launch of their apoptosis.
- The cascade of chemical reactions that activate coagulation and inflammatory response, leading to "hemorrhagic" tumor necrosis.
- Inhibition of angiogenesis of tumors.
- Activation of immune system cells (T-cells and natural killers).

TNF-α and apoptosis



TNF-α - high efficiency

- In 1992 in Russia, a new genetically engineered non-toxic tumor necrosis factor-α in combination with thymosin-alpha1 has been created (Professor Shmelev V. A.)
- **Refnot** is a recombinant -tumor necrosis factor-alpha + thymosin-α1, consisting of 185 amino acid residues, the last 28 of them on the C-end are the sequence of thymosin - α1.

Studies of TNF in the world today

targeted drug

- A phase I study of NGR-TNF, a novel vascular targeting agent, in patients with refractory solid tumors (EORTC 16041). Clin Cancer Res. 2010 A. Heerschap, (201 cycle, 69 patients, 27 patients – stabilisation of diseases, MRI – the damaging effect on blood vessels in tumors 2 hours after the beginning of the introduction has been proven)
- Vascular effects of the vascular targeting agent NGR-hTNF in patients with advanced solid cancer: A dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) EORTC study. H. Van Laarhoven, J Clin Oncol 28, 2010
- The novel direct-acting vascular targeting agent NGR-TNF exerts *in vivo* antitumor activity by inducing endothelial and tumor cell death in the absence of proangiogenic bone-marrow derived cell recruitment. Paola Di Matteo, *Cancer Research*: April 15, 2012;

Studies of TNF in the world today

combination with chemotherapy

Two doses of NGR-hTNF in combination with capecitabine plus oxaliplatin in colorectal cancer patients failing standard therapies. Mammoliti S, Ann Oncol. 2011 (a comparison of the effectiveness of high and low doses, the effect of the previously received these drugs)

- Defining the optimal biological dose of NGR-hTNF, a selective vascular targeting agent, in advanced solid tumours.

  Gregorc V, Eur J Cancer. 2010 (эффект в монотерапии).

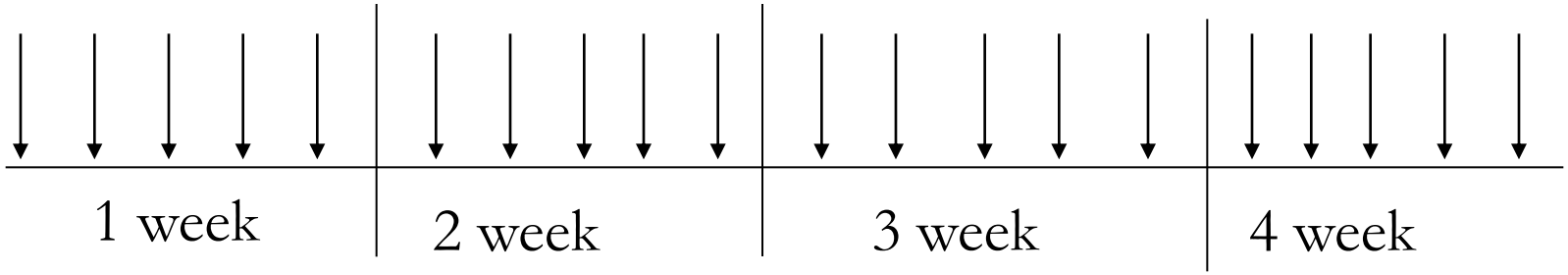
REFNOT – extension of indications

- **Objective**: Evaluation of the clinical efficacy and tolerability of Refnot in monotherapy and in combination with chemotherapy in patients with metastatic melanoma and study of the indices of immunity during treatment
- N.N. Blokhin RCRC of the RAMS.

15 patients with chemoresistant disseminated melanoma
The average age was 53.5 years

(Refnot in monotherapy)

Refnot 200.000 ME a day 5 days a week



Objective response – not marked

Stabilization - (4) 26,6%

N.N. Blokhin RCRC of the RAMS **(Abramov M.E. and co. 2010)**

The treatment regimen of patients with disseminated melanoma (Refnot + Polychemotherapy)

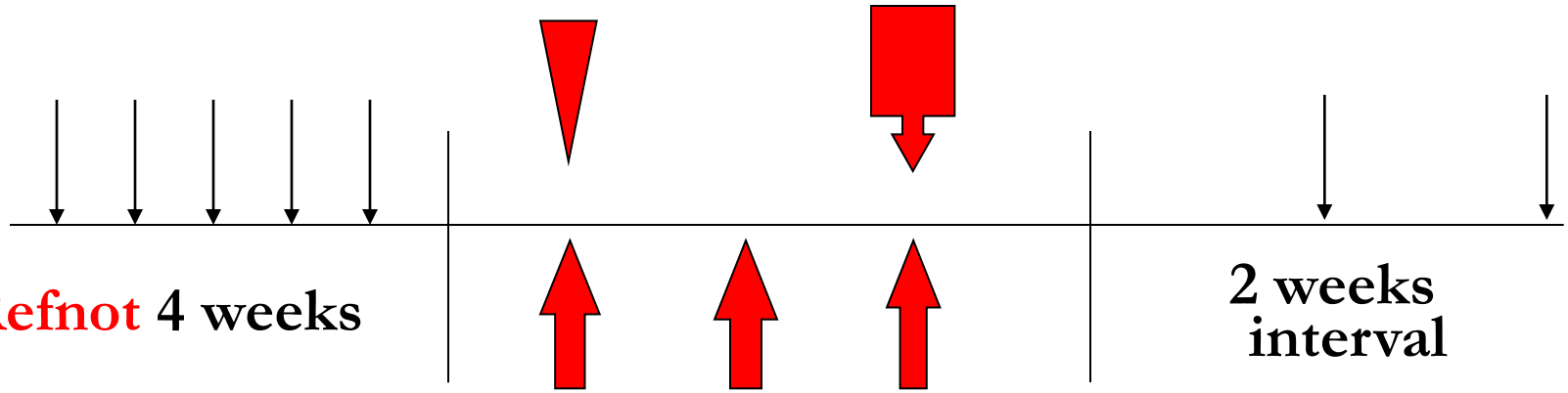
N.N. Blokhin RCRC of the RAMS **(Abramov M.E. and co. 2010)**

REFNOT 200.000 ME 5 days a week – 28 days

DACARBAZINE 250 mg/m^2 intravenously 1-3 дни

LOMUSTINE 80 mg/m^2 only one day of the course through 3 hours after the administration of dacarbazine.

CISPLATINE 80 mg/m^2 intravenously on the 3rd day of the course with pre-hydration after 1 hour after dacarbazine.



Effectiveness of regimen
Refnot+ chemotherapy

- 33 patients included (they have not previously received drug treatment for melanoma dissemination)
- **Full effect** – 2 (6%) patients
- **Partial effect** – 8 (18,2%) patients
- **Disease stabilization more than 3 months**. – 10 (30,3%)
- **Disease control** - 20 (54,5%)
- **Polychemotherapy (historical control)** – 20-25%

Study is continued ….

Side effects (Refnot)

Safety was assessed on a scale of toxicity (CTC), version 3:

- Asthenia 1– 2 degree
- Flushing of the skin and pain at the injection site – 1 degree
- Flu-like syndrome - 1 degree
- Повышение печеночных ферментов -1 degree
- Increased creatinine levels -1 degree
- Headache - 1 degree.

Характерны для всех цитокинов, хорошо контролируются и клинически не значимы

Clinical example 1

Patient K., 61

- **Diagnosis:** Melanoma of skin of back T2bN0M0. II A stage. Surgical treatment at 2006. Progression of disease at february, 2011: multiple lung metastases.
- **Anamnesis:** 04.2005 - excision of melanoma.
- 06.2006 Excision of melanoma of the skin of the back. No special treatment is received. At follow-up examination at 02.2011 they revealed multiple metastases to the lungs to 1.0 cm.
- **03.2011 – 12.2011 4 courses of chemoimmunotherapy:** Refnot + Dacarbazine + Lomustine + Cisplatine.
- **Examination after 2 courses (CT of the chest) –** full answer. **Examination after 4 months courses (CT of the chest)** : full effect persists.
- Further observation – up to 12.2013 - Effect lasted for 2 years.

Clinical example 2

Patient I., 48

Diagnosis: Disseminated melanoma of skin of right foot with multiple metastases to the mesentery of the small intestine (with verification of the diagnosis).

Anamnesis: 06.2003 - excision of melanoma of the skin of the right foot, the Duquene operationon the right side, further adjuvant immunotherapy for 12 months.

09.2009 The removal of metastases in the mesentery of the small intestine (10 cm) with resection of the small intestine. During the control of the CT 10. 2010 g.- multiple metastases in the mesentery of the small intestine (2.6 x 2.1 cm).

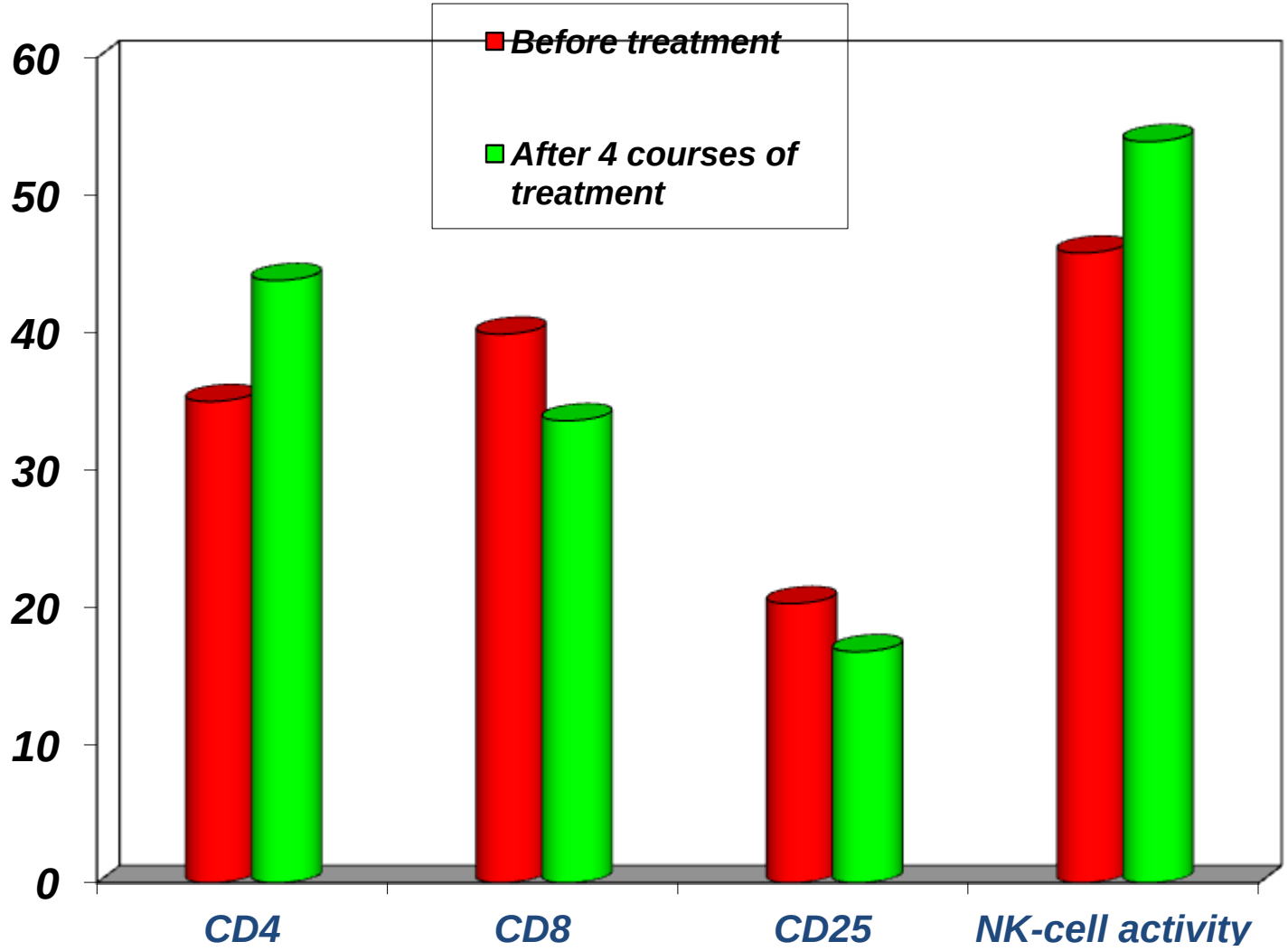
11.2010 - 08.2011 – 6 courses of polychemoimmunotherapy: Refnot + Dacarbazine + Lomustine + Cisplatine.

After 6 courses – full effect **by PET/CT of** 09.2011.: no metastates are determined.

Further follow-up.

03.2013g survey. - 18 months without symptoms of the disease.

Changes in the proportions of some lymphocyte subpopulations in all patients with melanoma after treatment with Refnot



TNF-α

- **REFNOT (TNF-α)** is registered by pharmacological RF Ministry of Health Committee in 2012 for a combined drug treatment of disseminated melanoma.
- **In USA and Europe TNF-α medicine is not developed for clinical use.**

Prospects

- Use of TNF-α as monotherapy in the treatment of various malignant tumors
- Use of TNF-α before chemotherapy to treat various malignancies in order to significantly enhance the cytotoxic effect of cytostatic agents (lung cancer, gastric cancer, colon cancer, ovarian cancer)

Prospects of systemic TNF- α therapy

1. **IFN-γ и (TNF-α) - related apoptosis-inducing ligand (TRAIL).**
2. **Adjuvant TNF-α - therapy.**
3. **Recombinant slow acting TNF-α - derived peptide.**

All the three studies of systemic TNF-α- therapy are currently undergoing the experimental investigation.

Cancer Biol Ther. 2014 Sep 1; 15(9): 1226–1238. Genetically engineered Newcastle disease virus expressing interleukin-2 and TNF-related apoptosis-inducing ligand for cancer therapy. Fu-Liang Bai, Yin-Hang Yu, Hui Tian etc.

Cancer Immunol Immunother. 2014 Sep;63(9):901-10. Preclinical evaluation of IL2-based immunocytokines supports their use in combination with dacarbazine, paclitaxel and TNF-based immunotherapy. Pretto F, Elia G, Castioni N, Neri D.

Sci Rep. 2015; 5: 13595. A novel recombinant slow-release TNF α-derived peptide effectively inhibits tumor growth and angiogensis. Yi Ma, Shaojun Zhao, Shutao Shen, etc.

Thank you for attention