



NRC Institute of Immunology FMBA of Russia

Novel immunotherapy approach for melanoma based chitosan derivatives

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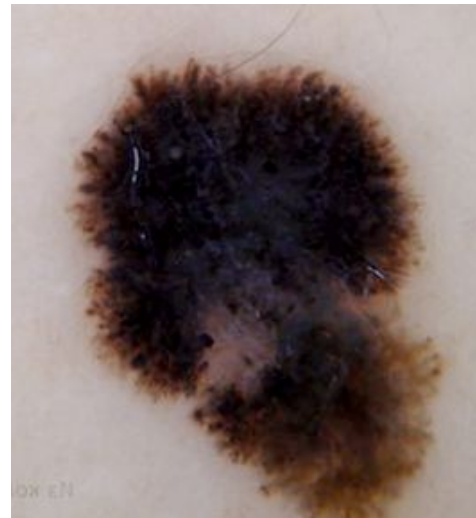
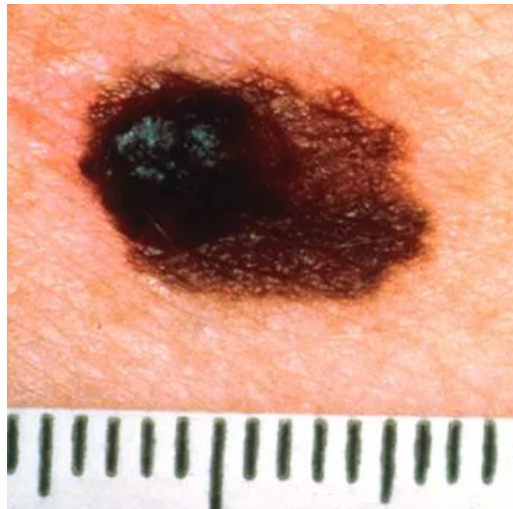
Definition

Melanoma is a type of skin cancer that develops from the pigment-producing cells known as melanocytes.

Melanomas typically occur in the skin but may rarely occur in the mouth, intestines or eye.

Typically, people are cured if metastatic spread has not occurred. For those in whom melanoma has spread, immunotherapy, biologic therapy, radiation therapy or chemotherapy may improve survival.

Five-year survival rates in US 99% (localized), 25% (disseminated). Therefore, melanoma is one of the most dangerous and insidious type of cancer.



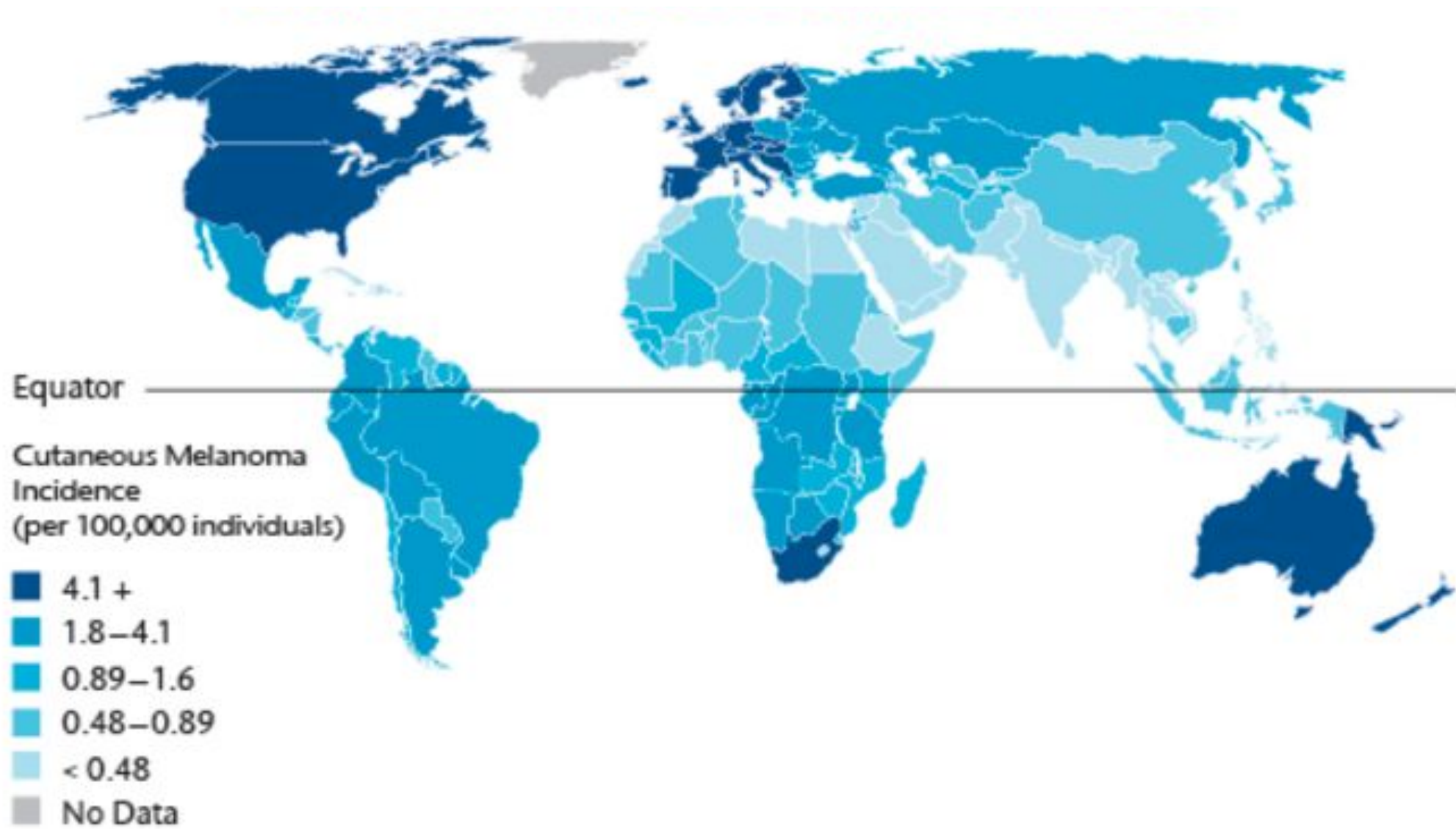


Figure 1. Variation in melanoma incidence

STAGES OF MELANOMA CANCER

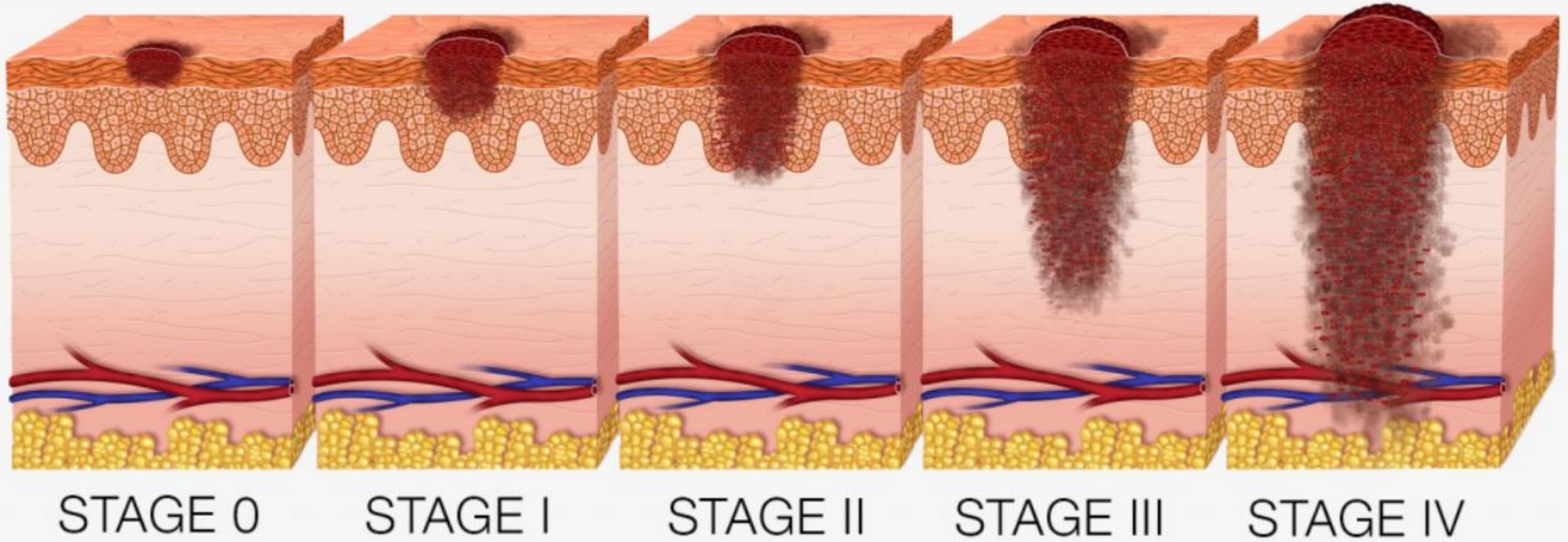


Figure 2. Clark's melanoma invasion classification

Revelance

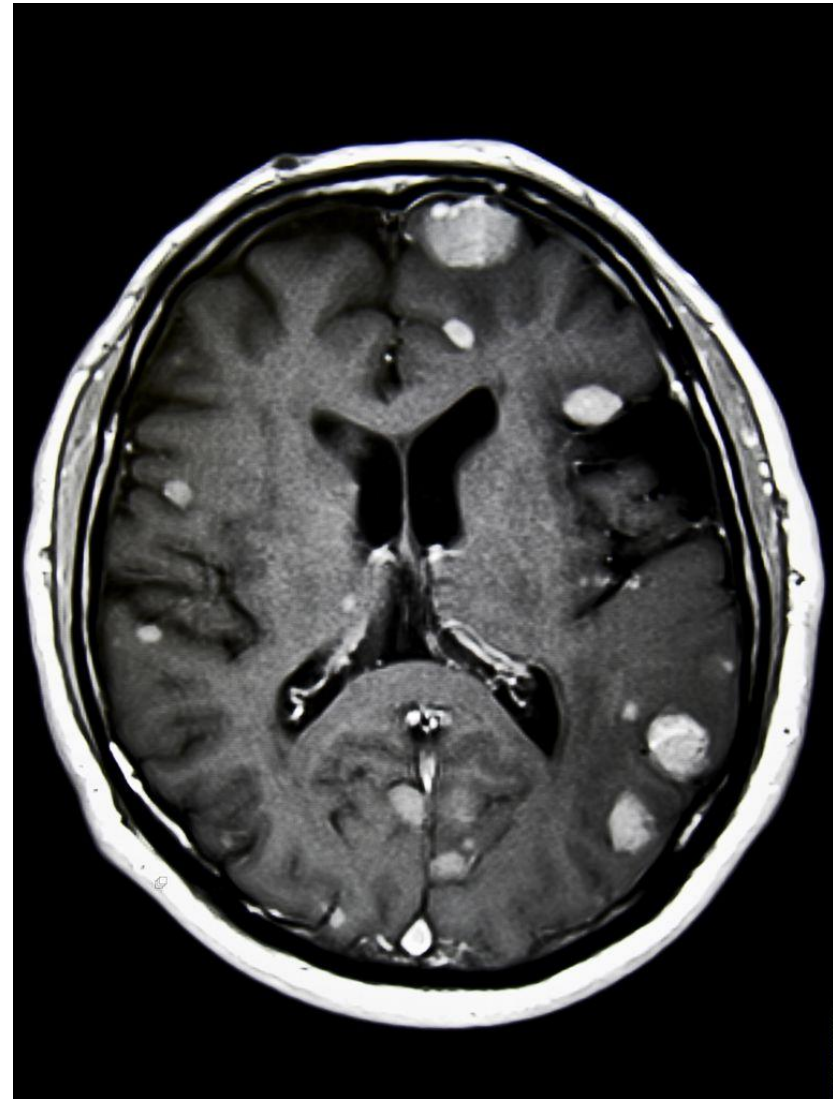
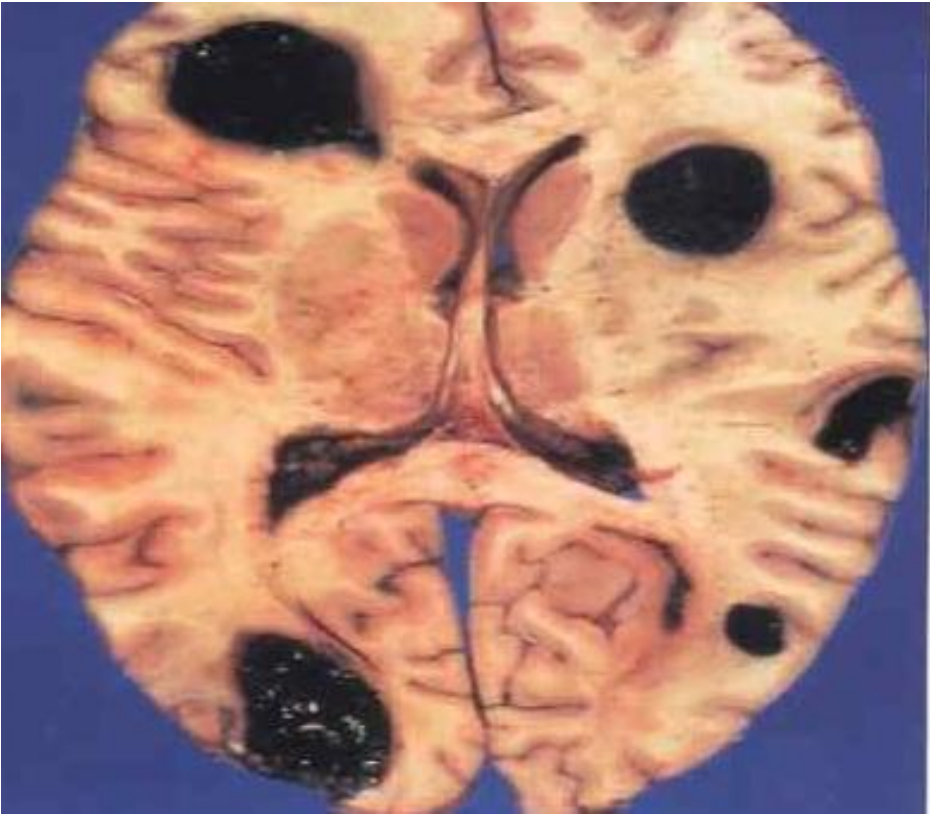


Figure 3.
Melanoma
metastases

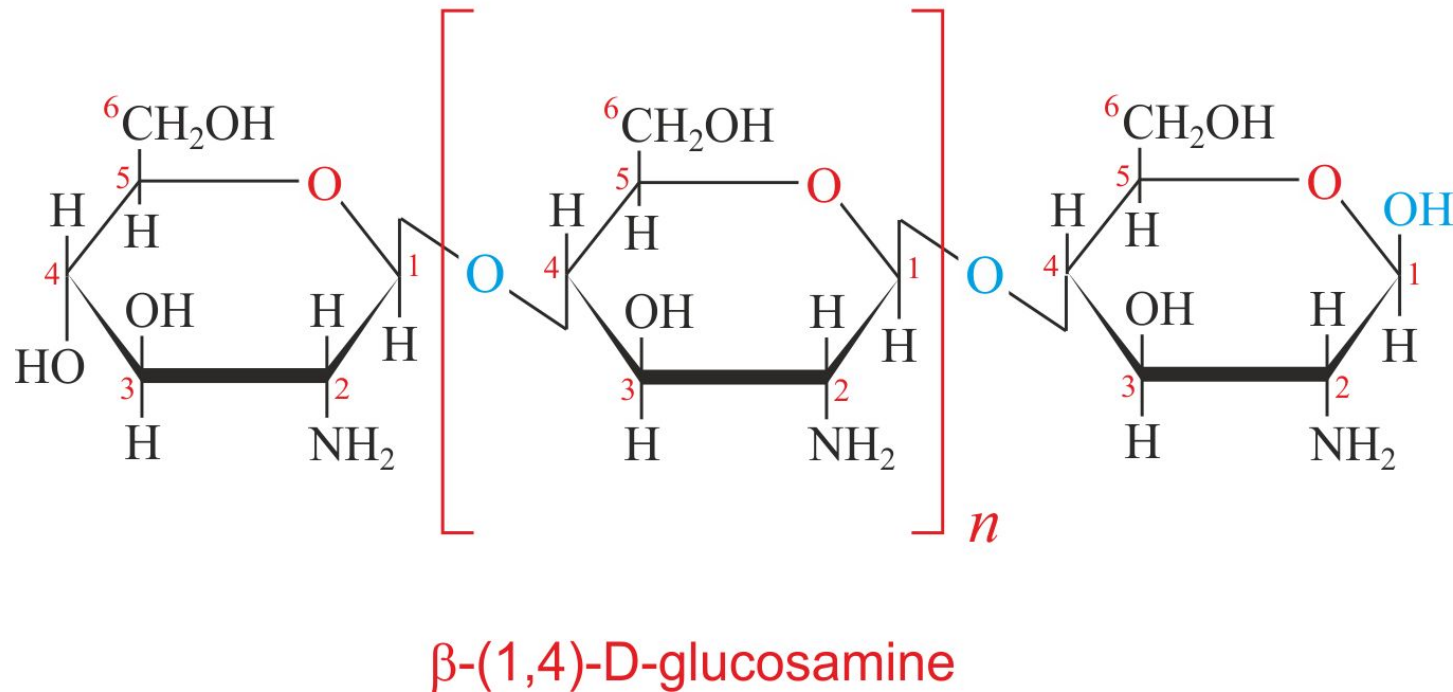
Revelance



Figure 4.
Melanoma metastases

Chitosan properties

Chitosan



Chitosan /'kaɪtəsæn/ is a linear polysaccharide composed of randomly distributed β -(1 \rightarrow 4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit).

It is made by treating the chitin shells of shrimp and other crustaceans with an alkaline substance, such as sodium hydroxide.

Figure 5. Chemical formula of chitosan

Chitosan nanoparticles

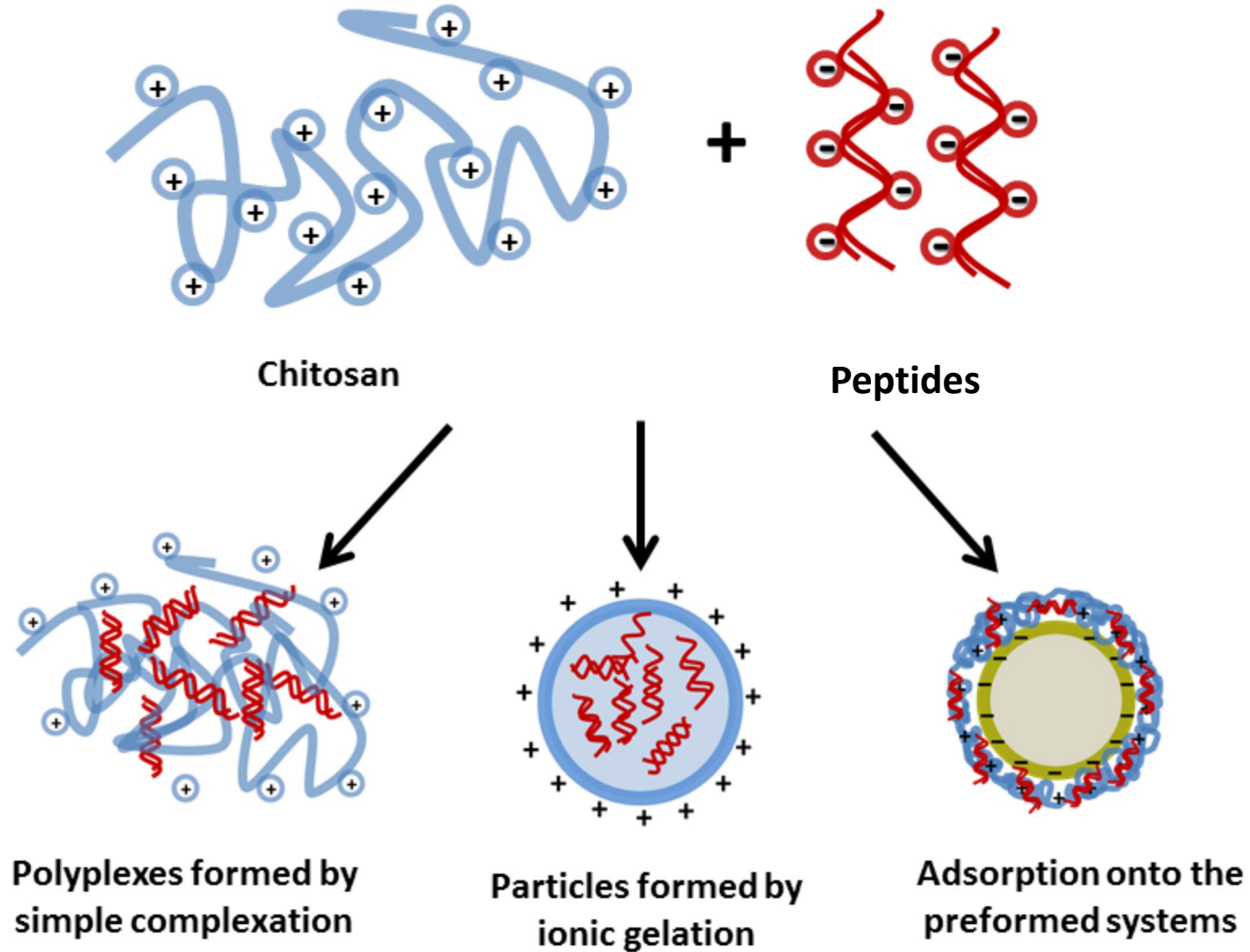


Figure 6. Chitosan nanoparticles

Chitosan nanoparticles

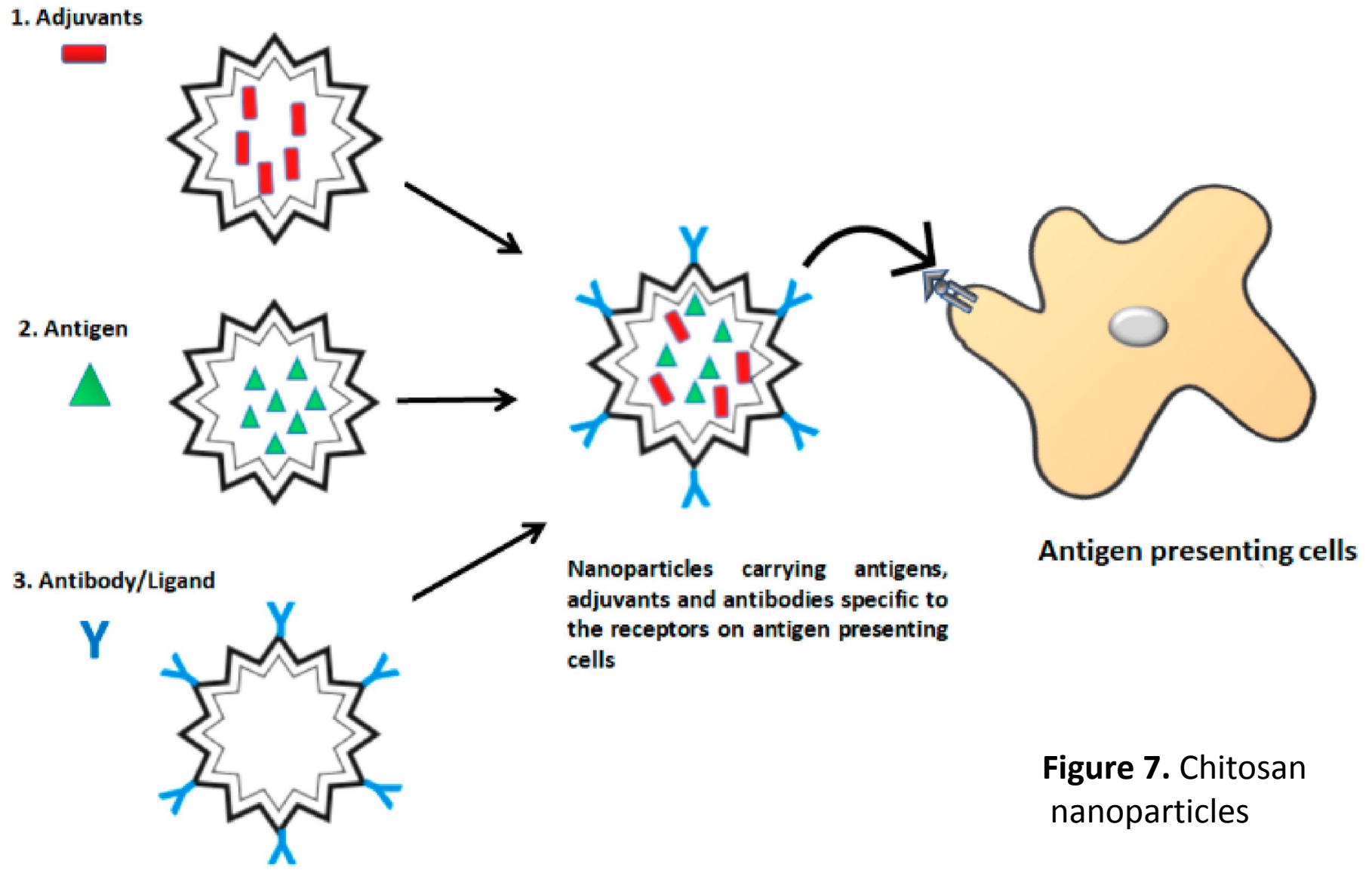


Figure 7. Chitosan nanoparticles

Chitosan properties

Biological properties of chitin and chitosan

- Non-toxicity
- Biodegradability
- Biocompatibility
- Citocompatibility
- Antimicrobial activity
- Anticholesterolemic activity
- Antioxidant activity
- Anti-inflammatory action
- Analgesic action
- Haemostatic action
- Mucoadhesion
- Angiogenesis stimulation
- Macrophage activation
- Granulation and scar formation
- Adsorption enhancer

Therefore, this substance can be used to create a series of drugs.

We can use for the treatment of melanoma:

Chitosan based vaccines

Modified delayed release preparations of cytokines

Various targeted drug carriers

Purpose of the study

Creation of a complex of drugs for immunotherapy of melanoma

Research objectives

1. Development methods of synthesis, purification and standardization of highly purified chitosan. (*chemical part*)
2. Getting set of drugs for testing.
3. Estimate the toxicity of drugs at *in vitro* experiments (MTT test) and *in vivo*.
4. Determine the allergic activity of chitosan derivatives at *in vitro* experiments and *in vivo*. (*allergic part*)
5. Estimate the immunostimulating properties of highly purified chitosan derivatives. (*immunology part*)
6. Explore the benefit of using nanoparticles and covalently bonded chitosan derivatives with interleukins; chitosan-based vaccine structures and targeted platforms to increase the therapeutic efficacy of therapy melanoma at *in vivo* experiment.

Chemical part of experiments

Methods for drug standardization:

1. Mass spectrometry (MS)
2. High-performance liquid chromatography (HPLC)
3. Infrared spectroscopy (IR spectroscopy)
4. Viscometry
5. Dynamic light scattering (DLS)
6. Electrophoresis

The main parameters of chitosan, which determine the immuno- and allergic activity:

1. Molecular weight
2. Weight average distribution
3. Degree of deacetylation
4. Solubility at physiological pH
5. Degree of contamination with protein and peptide fractions
6. Degree of endotoxin contamination and other TLR agonists

Mass spectrometry (MS)



Figure 8. Photo of Mass spectrometr

Mass spectrometry (MS) is an analytical technique that is used to measure the mass-to-charge ratio of ions. The results are typically presented as a mass spectrum, a plot of intensity as a function of the mass-to-charge ratio.

Methods M.-s. are used in various fields: in the nuclear industry, medicine, biology, metallurgy, geology, space. research, when conducting elemental analysis of high-purity substances and determining the absolute amount of substances by isotopic dilution, when controlling the quality of food and water, etc.

These methods are used to analyze complex mixtures and determine the structure of molecules.

Allergic part

Methods for estimate allergic potential:

Histamine release in heparinized whole blood

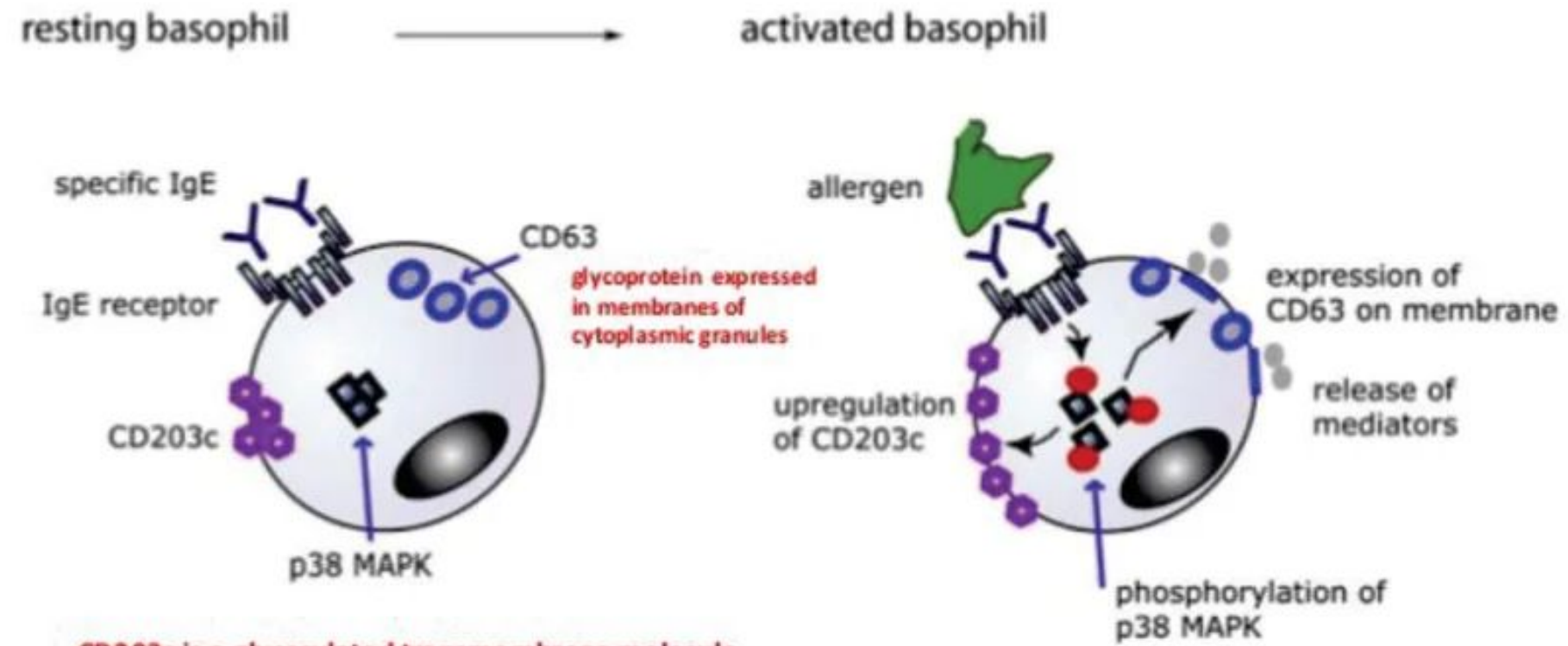
Basophil activation test

Lymphocyte activation test

Experiments in vivo

Basophil activation test

Basophil activation markers: CD63 & CD203c



CD203c is a glycosylated transmembrane molecule
Basophils express low level of CD203c, which is increased
with basophil activation and may correspond to piecemeal degranulation

Figure 9.
Scheme of
BAT

Immunology part

Methods for estimate immunological potential:

Determine the activity of endocytosis and pinocytosis by fluorescently labeled chitosan.

Fix the main parameters of chitosan that determine the immunoactivity (include endotoxin level).

Test dendritic cell activation.

Test T - cell activation.

In vivo experiments.

Last part of research

In vivo (mouse models) experiments of chitosan based complex for activity to melanoma

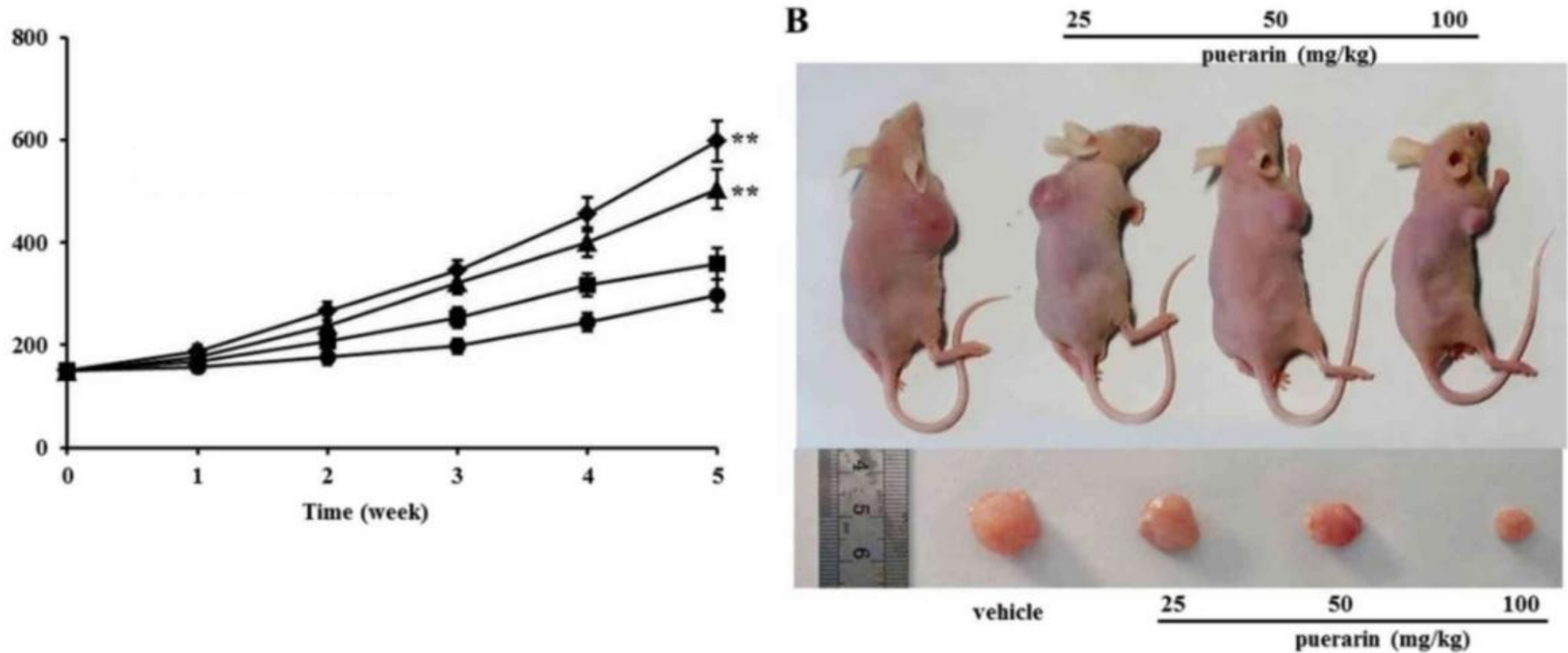


Figure 9. Scheme of in vivo experiments



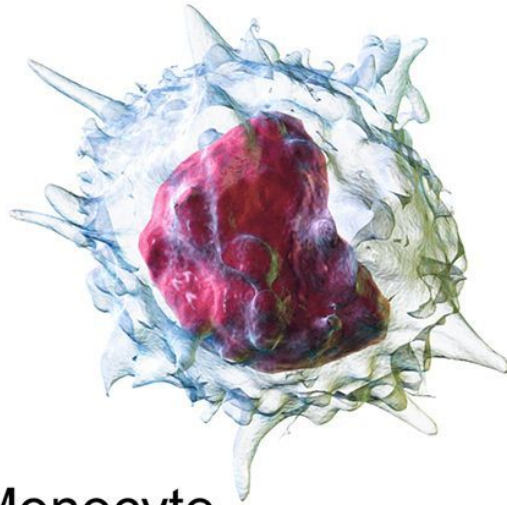
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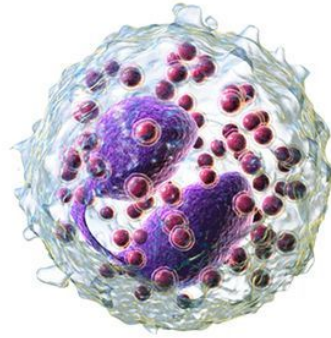
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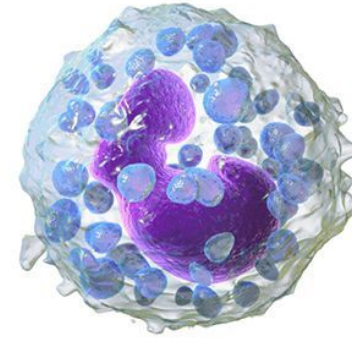
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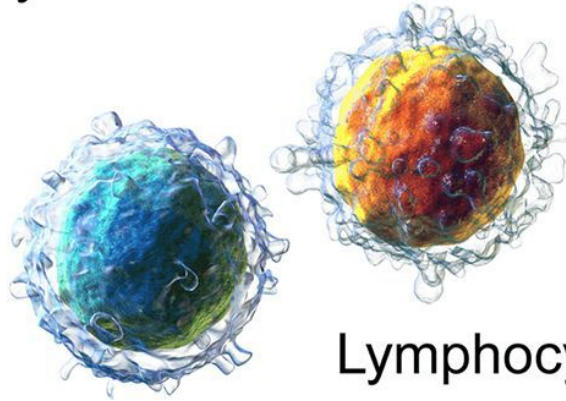
Monocyte



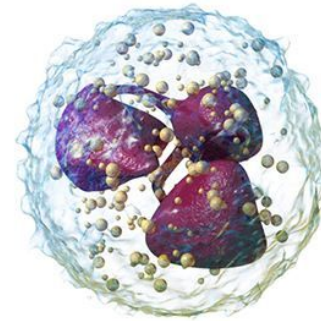
Eosinophil



Basophil



Lymphocytes



Neutrophil

Thank you for attention