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“Investing in Sustainable Development”

nuclear physics

Extreme Light Infrastructure-Nuclear Physics (ELI-NP) - Phase II

IFIN-HH

Combining immunotherapy with radiology, new pathways of research supported by particles provided by the high-power laser system at ELI-NP

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Victor Babeș Institute Bucharest
2-4 November 2023

Historic Remarks, Immunotherapy & Radiology

- Immunology & Radiology are strongholds in cancer therapy, emerging almost in parallel, **but** almost naturally on very different pathways
- Radiology
  - X-rays 1895 Röntgen, just 3 days (!) after application of X-rays for cancer treatment (Ludlam)
  - Nuclear decay (Becquerel 1896) $\rightarrow$ Radiumtherapy (Curietherapy) $\sim$ 1900
  - Protontherapy: Wilson (1946), first treatments 1950's, prominence 1990's (accelerator-based)
  - Boron Neutron Capture Therapy (BNCT), Sweet MIT (1954), MIT research reactor [Led81]
- Immunotherapy (tremendous recent successes! - Thank You!)
  - Purposeful viral inoculation to prevent smallpox disease, 3rd century B.C. (China), Jenner (1798)
  - Cancer Immunotherapy: Coley, 1st harness immune system for bone cancer treatment (1891) 'Coley's Toxins'
  - 'The Breakthrough': J. P. Allison and T. Honjo: Cancer therapy by inhibition of negative immune regulation Keytruda (book by C. Graeber [Gra18])
- Radioimmunotherpay (RIT) bringing it together (Goldenberg (1978) [GDK$^+$78]), personalized treatment
  - Personalized cancer treatment, combining radiation therapy with the precise targeting ability of immunotherapy
  - First commercial: Ibritumomab tiuxetan (FDA) in 2002 (types of non-Hodgkin lymphoma)
  - Our idea: Combining BNCT with low-energetic neutrons provided by ELI-NP with allogenic $\gamma\delta$ CAR-T cells, loaden with boron-nanoparticles or any other favorable isotope (potentially radio-tracer)

Core Idea & Nobel Prize Ceremony 2018

Core to our idea:

- Immunotherapy and the use of $\gamma\delta$ CAR-T cells as effective delivery agents ('nanorobots') for sufficient amounts of radiotherapeutic isotopes
- High precision delivery of epithermal (slow) neutrons from a pulsed source for Boron Neutron Capture Therapy (BNCT), herein high-power laser-plasma systems (HPLS), *e.g.* the 1 PW and 10 PW flagship installations at ELI-NP will become game changers (G. Mourou & D. Strickland)

Fig. – The 2018 Nobel Prize Award Ceremony

Boron Neutron Capture Therapy (BNCT)

- redBNCT is scalable = the higher the boron concentration the less demand on neutron flux on patient
- BNCT is in clinical phase trials, worldwide $\sim$ 3000 treatments (Jp,China,USA,Fin,Swe) [KMK+09]
- As of 2023 33! accelerator-driven centers build
- ^{10}B is a stable component of $^{\mathrm{nat}}\mathrm{B}$, $\sim$ 20% abundance, , non-radioactive, $m(70\,\mathrm{kg})_{\mathrm{body}} \sim 1.8 \times 10^{-5}$ kg
- Nuclear reaction:

$$^{10}\mathrm{B} + \mathrm{n_{epi}} \rightarrow ^{11}\mathrm{B}^* \rightarrow ^{7}\mathrm{Li} + \alpha \;\&\; 2.78\,\mathrm{MeV} = 2.8\,\mathrm{MeV}\ \mathrm{released}$$

- Capture process has for thermal and epithermal neutrons ($\sim$ 0.025 eV) a very high cross-section $\sigma = 3850$ mb [COH+06]- That's good, very good
- Per cell only 20 fg needed in cell for 99.99% of destruction if cell exposed to 10^{12} N_n(epi), for 0.1 nGy in **total** one gets 0.3 µGy **per** boron-loaden cancerous cell! Dose rate: $\leq$ 70 Gy will allow $V_{\mathrm{sol.tum.}} \sim \mathrm{cm}^3$
- Reaction products are themselves ions! So, BNCT is a short-range ion therapy $\longrightarrow$ BNCT is selective!
  - DNA damage by the electronic energy loss S_{e^-} of $\alpha = {}^{4}\mathrm{He}$ and $^{7}\mathrm{Li}$ ions
  - DNA damage only localized in cellular dimension, big big **+** compared to ion-based and X-ray-based therapies, NO surrounding helathy tissue is harmed
- Only acts within the malignant cell, or in its direct vicinity if IDEAL SELECTIVE CARRIER can be found (range: 5 µm to 8 µm) $\sim \varnothing$(cell), Minimal radiation damage to surrounding cells

BNCT, Selective & Steerable!

- As ^{10}B is stable, the nuclear reaction can be fully controlled with *e.g.* step-wise switch on/off function AT ANY OPPORTUNE TIMING and steered by clinicians. No background radiation after treatment!
- Find an ultra-precise delivery agent: genetically modified $\gamma\delta$ CAR-T cells (allogenic)
  - Infusion of boron nanoparticles together with cytotoxins to enhance the therapeutic function
  - Most commonly used: boronophenylalanine (BPA), limited enhancement cancer-to-healthy: 4-to-1

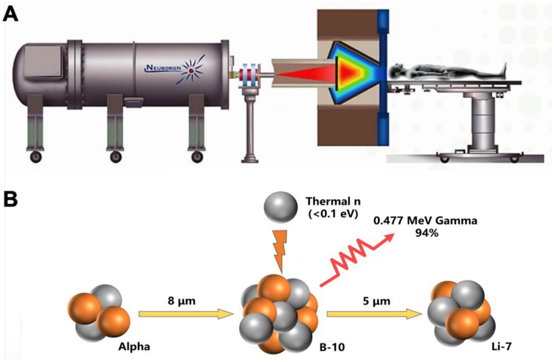


Fig. – Schematics of BNCT [DYB+22]

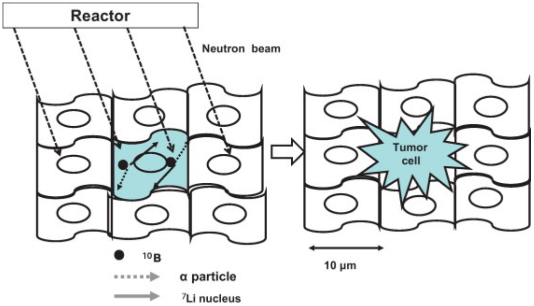


Fig. – Boron implanted cancerous cell in healthy cell environment [YTFH19]

In Vitro Sonoporation of $\gamma\delta$ CAR-T cells with (boron) nanoparticles

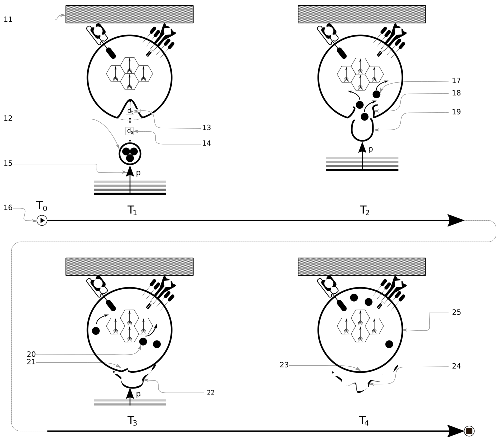


Fig. – Sonoporation of boron nanoparticle with microbubbles

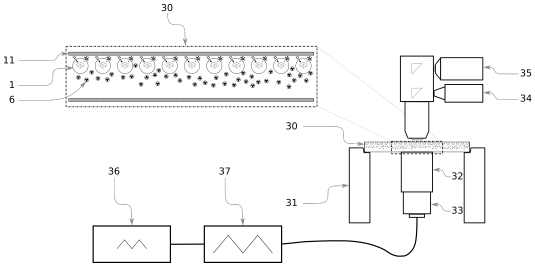


Fig. – Sketch of sonoporation scheme with ultrasound and microscope surveillance

In Vitro Sonoporation of $\gamma\delta$ CAR-T cells, Rayleigh-Plesset Formula

Rayleigh-Plesset Equation

$$R(t)\frac{d^2R(t)}{dt^2} + \frac{3}{2}\left(\frac{dR(t)}{dt}\right)^2 + \frac{4\nu_L}{R(t)}\frac{dR(t)}{dt} + \frac{2\gamma}{\varrho_L R} + \frac{\Delta P(t)}{\varrho_L} = 0, \quad (1)$$

- Finding optimum parameters of pressure $P(t)$, frequency f, and bubble diameter
- Softest possible approach to sonoporation NOT to destroy or compromise complex T cells
- K. M. Spohr *et al.* Rom. Rep. Phys 75, 601 (2023) (successful PED Grant)

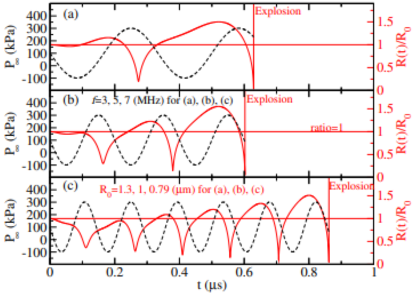


Fig. – Rayleigh-Plesset equation for SonoVue bubbles

f_{res} / MHz	R_0^{min} / μm	R_0^{max} / μm	R_0^{opt} / μm	T_{expl} / μs
3	0.87	2.28	1.29	0.63
4	1.02	1.19	1.08	0.73
5	0.90	1.04	0.99	0.59
6	0.83	0.90	0.88	0.67
7	0.77	0.80	0.79	0.86
8	0.718	0.719	0.719	3.00

Table 1.

Fig. – Summary of sonoporation simulations for SonoVue bubbles

Boron-loaden $\gamma\delta$ CAR-T cells, envisaged *modus operandi*

- Genetically modified, allogenic-produced!, boron-loaden $\gamma\delta$ CAR-T cells find their way to cancerous cells for attack, initially bloodbourne
- ONLY docking to malignant cells due to receptors, but NOT docking to healthy cells, due to the customized receptors
- Infusing cytotoxins & boron nanoparticles ONLY into malignant cells *via* Kiss of Death
- Due to the localized nature of the boron decay, approaching the malignant cell is sufficient for BNCT to work (very different from mRNA treatment!)
- Control and survey of BNCT process *via* pulsed neutron impact from ultra-fast, switchable neutron source provided by an HPLS (ELI-NP)

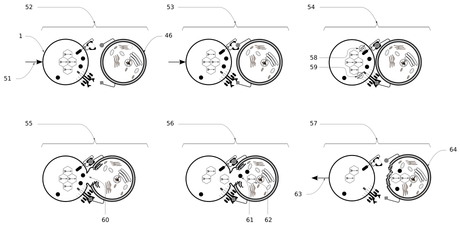


Fig. – *Modus Operandi* of boron-loaden $\gamma\delta$ CAR-T's

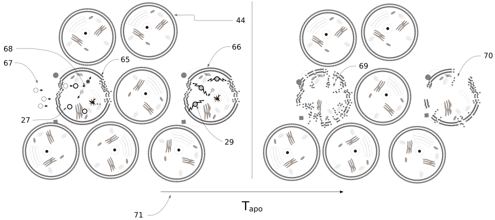


Fig. – Boron-loaden cancer cells in BNCT

Extreme Light Infrastructure - Nuclear Physics (ELI-NP) in Bucharest

- A 320 m€ investment by EU our 10 PW, HPLS with highest peak power laser in the world!
- Ideal provider of strong, pulsed neutron sources for *prima faci* studies, with 10 PW and 1 PW stations in the future (dedicated neutron source program)
  - 10 PW? = 250 J of energy in laser light delivered in ultra-short timespan 25 fs
  - Accelerating protons to $\sim$ 65% speed of light ($\sim$ 1 GeV) $\longrightarrow$ efficient neutron production
  - Laser-induced neutrons come with short pulse durations (μs) and small source sizes μm^3 $\longrightarrow$ temporal and spatial control, for optimizing the neutron fluxes for BNCT

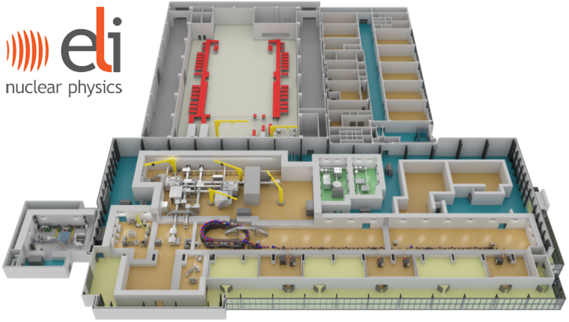


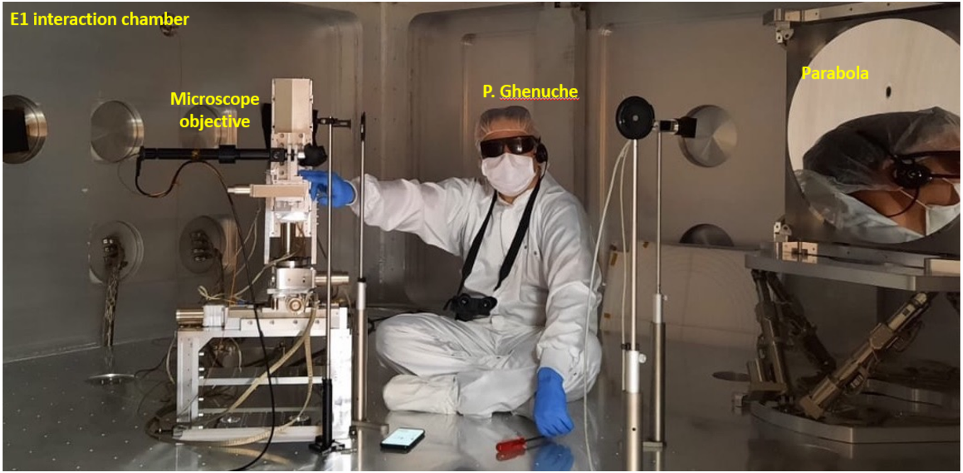
Fig. – Layout of the ELI-NP facility in Bucharest-Măgurele

ELI-NP, Impressions - 10 PW HPLS commissioning at E1 (2023)

ELI-NP, Impressions - 10 PW HPLS commissioning at E1 (2023)

10 PW E1 experimental area commissioning (from 26 Sept 2022)

Laser beam alignment and focal spot check



ELI-NP: High-intensity bursts of thermal/epithermal neutrons

- Use of magnetic neutron lenses

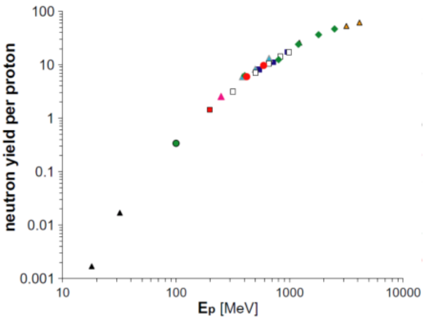


Fig. – Neutron yield per proton reaction as f(E_p)

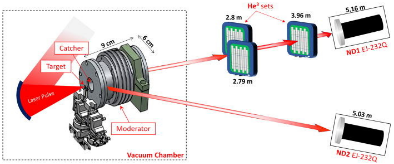


Fig. – Compact neutron moderator, a special feature of HPLS [MAA+17]

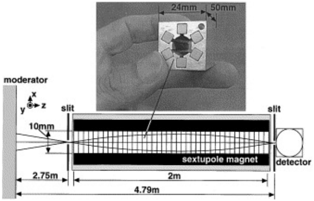


Fig. – Example of a magnetic neutron lense [SOS+00]

Sketch of future treatment, Multidisciplinary approach is the key!

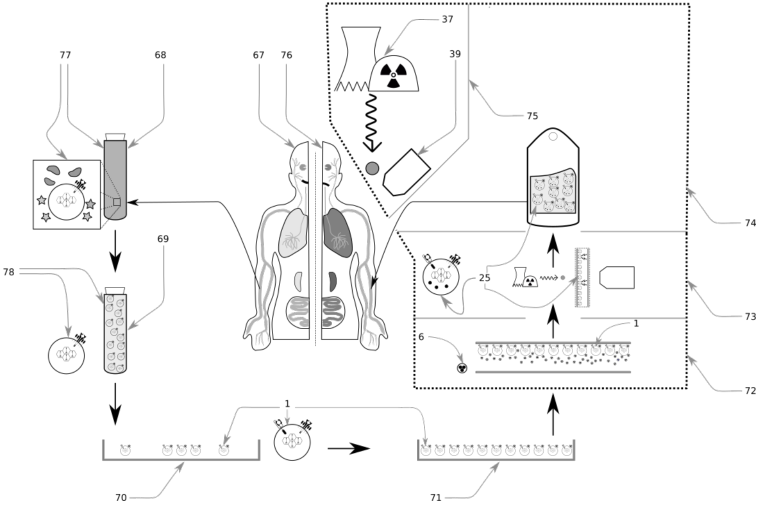


Fig. – Treatment pathway with gen-manipulated & allogenic-produced boron-loaden $\gamma\delta$ CAR-T cells

Strategy of research program

- Efficacy study at any *in vitro* aspect of the $\gamma\delta$ CAR-T cell supported BNCT
  - Sourcing and expansion of allogenic-produced $\gamma\delta$ CAR-T cells (-80° fridge network cluster?)
  - Ultrasonic boron-loading process: $P(t)$, $f(t)$, $N(\gamma\delta$ CAR-T$)$, $N(^{10}\mathrm{B})$ & type of transducer, selection of microbubbles (SonoVue), Protocols and Quality Control
  - Sonoporation process (loading efficiency, $\gamma\delta$ CAR-T cell survival, $\gamma\delta$ CAR-T cell functionality)
  - Efficiency of *in vitro* production
  - Efficiency of 'Kiss of Death' with respect to boron transfer
  - Suitability of *Modus Operandi* of boron-transfer to cancer cells only *in vitro* model
  - Efficiency of BNCT for cancerous cells in a melee of malignant/healthy cells *in vitro* model
  - Cost-effectiveness (A big + : allogenic-sourced $\gamma\delta$ CAR-T cells)
  - Step towards 'mouse-model'?
  - Further Combination Therapies?
  - First cancer targets of bloodbourne nature, after that, progressing to solid
  - Romania is ideally placed to spearhead this research due to having the key competencies (institutes, facilities & human (power)m resource (young researchers from a pristine educational system of highest standards!))
- Multumesc, Dankeschön, & Thank You

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