Final scientific report

Project title: Identifying of cytogenetic and immunological markers to estimate the risk of radiation-related chronic side effects of prostate cancer patients
Project leader: Géza Sáfrány
Project ID: 124879
Report prepared by: Katalin Balázs (NPHC), Zsolt Jurányi (NIO)

Publications:

- Eszter Persa, Tünde Szatmári, Géza Sáfrány, Katalin Lumniczky: ***In Vivo Irradiation of Mice Induces Activation of Dendritic Cells***, 08.2018, Cancers, International Journal of Molecular Sciences; (doi: 10.3390/ijms19082391)

- Katalin Balázs, Enikő Kis, Christophe Badie ,Enikő Noémi Bogdándi ,Serge Candéias, Lourdes Cruz Garcia, Iwona Dominczyk, Benjamin Frey, Udo Gaipl, Zsolt Jurányi, Zsuzsa S. Kocsis, Eric Andreas Rutten, Géza Sáfrány, Piotr Widlak, Katalin Lumniczky: ***Radiotherapy-Induced Changes in the Systemic Immune and Inflammation Parameters of Head and Neck Cancer Patients,*** 06.09.2019, Cancers (https://doi.org/10.3390/cancers11091324)

Szatmári T, Persa E, Kis E, Benedek A, Hargitai R, Sáfrány G, Lumniczky K: ***Extracellular vesicles mediate low dose ionizing radiation-induced immune and inflammatory responses in the blood.*** Int J Radiat Biol. 2018 Mar 29:1-11. https://www.tandfonline.com/doi/full/10.1080/09553002.2018.1450533

Hargitai R, Kis D, Persa E, Szatmári T, Sáfrány G, Lumniczky K: Oxidative Stress and Gene Expression Modifications Mediated by Extracellular Vesicles: An In Vivo Study of the Radiation-Induced Bystander Effect, Antioxidants 2021, 10(2), 156, 2021; https://www.mdpi.com/2076-3921/10/2/156

- Katalin Balázs, Lilla Antal, Géza Sáfrány and Katalin Lumniczky: ***Blood-Derived Biomarkers of Diagnosis, Prognosis and Therapy Response in Prostate Cancer Patients*** 13.04.2021; Journal of Personalized Medicine; (https://doi.org/10.3390/jpm11040296)

- Katalin Balázs, Zsuzsa Kocsis S, Péter Ágoston, Kliton Jorgo, László Gesztesi, Göngyi Farkas, Gábor Székely, Zoltán Takácsi-Nagy, Csaba Polgár, Géza Sáfrány, Zsolt Jurányi, Katalin Lumniczky: ***Prostate Cancer Survivors Present Long-Term, Residual Systemic Immune Alterations***, 22.06.2022; Cancers (https://doi.org/10.3390/cancers14133058)

- Dávid Kis, Ilona Barbara Csordás, Eszter Persa, Bálint Jezsó, Rita Hargitai, Tünde Szatmári, Nikolett Sándor, Enikő Kis, Katalin Balázs, Sáfrány Géza, Lumniczky Katalin: ***Extracellular Vesicles Derived from Bone Marrow in an Early Stage of Ionizing Radiation Damage Are Able to Induce Bystander Responses in the Bone Marrow*** (2022); Cells; (doi: 10.3390/cells11010155)

- Farkas G, Kocsis ZS, Székely G, Dobozi M, Kenessey I, Polgár C, Jurányi Z.: ***Smoking, chromosomal aberrations, and cancer incidence in healthy subjects.***Mutat Res Genet

Toxicol Environ Mutagen. 2021 Jul; 867:503373. (doi: 10.1016/j.mrgentox.2021.503373) Epub 2021 Jun 18.

- Kocsis ZS, Farkas G, Bajcsay A, Kun-Gazda M, Lövey J, Ostoros G, Pócza T, Herein A, Ladányi K, Székely G, Markóczy Z, Takácsi-Nagy Z, Polgár C**,** Jurányi Z.: ***Chromosomal Aberrations in Blood Lymphocytes as Predictors of Respiratory Function After Stereotactic Lung Irradiation***. Front Oncol. 2022 Jan 27;11:829972. (doi: 10.3389/fonc.2021.829972)

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- Farkas G, Kocsis ZS, Székely G, Kenessey I, Polgár C, Jurányi Z. ***Spontaneous Chromosomal Aberrations in Lymphocytes and Development of Tumor in Hospital Workers***. Anticancer Res. 2022 Feb; 42(2):1059-1064. (doi: 10.21873/anticanres.15567

- **under submission:** Katalin Balázs, Zsuzsa Kocsis S, Péter Ágoston, Kliton Jorgo, László Gesztesi, Göngyi Farkas, Gábor Székely, Zoltán Takácsi-Nagy, Csaba Polgár, Géza Sáfrány, Zsolt Jurányi, Katalin Lumniczky: ***The acute and long-lasting effects of high-dose rate brachytherapy on the innate and adaptive immune system of prostate cancer patients***

Participation at scientific meetings between 2017-2022 (NIO):
2017
Gyöngyi Farkas, Csilla Pesznyák, Dalma Béla, Gábor Székely, Zsuzsa S. Kocsis, Tibor Major, Zsolt Jurányi, Csaba Polgár. Biological dose estimation for different photon beam qualities used in radiation oncology. 8th Alpe-Adria Medical Physics Meeting, 2017 május 25 -27, Novi Sad, Szerbia.

Gyöngyi Farkas, Csilla Pesznyák, Dalma Béla, Gábor Székely, Zsuzsa S. Kocsis, Tibor Major, Zsolt Jurányi, Csaba Polgár. Biological dose estimation for different photon beam qualities used in radiation oncology. International Conference on Advances in Radiation Oncology (ICARO2). Bécs, 2017 június 20–23.

Kocsis S. Zsuzsa, Ágoston Péter, Farkas Gyöngyi, Székely Gábor, Jorgo Kliton, Polgár Csaba, Jurányi Zsolt. Prosztata tumoros betegek háromféle sugárkezelésének összehasonlítása kromoszómaaberrációs és klinikai szempontok szerint. MST Kongresszus, 2017. május 18-20., Győr.

Jurányi Zsolt. Biológiai dozimetria. Klinikai sugárbiológia tanfolyam, 2017.10.04-06., Budapest.

Farkas Gyöngyi, Környezet, genetika és rák. Betegoktatási program, Országos Onkológiai Intézet, 2017 XXX.

Jurányi Zsolt. Teendők rákterápia alatt – a betegek szemszögéből. Betegoktatási program, Országos Onkológiai Intézet, 2017. Október 19.

Kocsis S. Zsuzsa. Sejtből is megárt a sok. Előadás és mikroszkópos játék, Kutatók éjszakája, 2017.09.29., Országos Onkológiai Intézet.

Jurányi Zsolt. Sugárterápia, sugárbiológia és citogenetika az ionizáló sugárzás hatása a kromoszómák szerkezetére és szerepe a daganatterápiában. BME Szent-Györgyi Albert Szakkollégium Kongresszus, 2017. április 7-8., Budapest.

2018

Timea Hülber, Zs. S. Kocsis, E. Kis, G. Sáfrány, Cs. Pesznyák. Overview of the performance parameters and unique features of a recently developed automatic micronucleus assay evaluation system. 44th European Radiation Research Congress, 2018. August 21-25, Pécs.

Gyöngyi Farkas, András Bajcsay, Gyula Ostoros, Zsolt Markóczy, Zsuzsa S. Kocsis, Márta Kun-Gazda, Gábor Székely, Dalma Mihály, József Lövey, Csaba Polgár, Zsolt Jurányi. Relationship between biodosimetry and respiratory function values in lung stereotactic radiotherapy patients. 44th European Radiation Research Congress, 2018. August 21-25, Pécs.

Gyöngyi Farkas, Zsuzsa S. Kocsis, Gábor Székely, Dalma Béla, Csilla Pesznyák, Tibor Major, Csaba Polgár, Zsolt Jurányi. Calculation of dose-response curves for in vitro biodosimetry using a linear accelerator either in FF or FFF modes. World Congress for Medical Physics and Biomedical Engineering, Prague, 2018. June 3-8, 2018.

Tímea Hülber, Enikő Kis, Zsuzsa S. Kocsis, Géza Sáfrány, Csilla Pesznyák Development of an image-processing based sample quality index for non-fluorescent micronucleus assay which offers extended robustness feature for automated scoring, EPRBioDose 2018 International Conference, 11-15 June 2018, München, Germany.

Kocsis S. Zsuzsa, Major Tibor, Mihály Dalma, Stelczer Gábor, Farkas Gyöngyi, Székely Gábor, Ágoston Péter, Jorgo Kliton, Gesztesi László, Polgár Csaba, Jurányi Zsolt. Chromosome aberration biodosimetry toin compareing three kind of prostate radiotherapy. 44th European Radiation Research Congress, 2018. August 21-25, Pécs.

Kocsis S. Zsuzsa, Ágoston Péter, Farkas Gyöngyi, Kun-Gazda Márta, Székely Gábor, Major Tibor, Mihály Dalma, Pesznyák Csilla, Stelczer Gábor, Jorgo Kliton, Gesztesi László, Polgár Csaba, Jurányi Zsolt. HDR, seed brachyterápia és külső prosztata sugárkezelés összehasonlítása biodozimetria és mellékhatások szempontjából. FIOSZ, 2018. Szeptember 14, Budapest.

Zsuzsa S. Kocsis, Péter Ágoston, Gyöngyi Farkas, Gábor Székely, Kliton Jorgó, Csaba Polgár, Zsolt Jurányi. Comparison of HDR, seed brachytherapy and teletherapy in the perspective of chromosomal damage and radiogen toxicities. World Congress for Medical Physics and Biomedical Engineering, Prague, 2018. June 3-8, 2018.

Kocsis S. Zsuzsa, Major Tibor, Mihály Dalma, Stelczer Gábor, Farkas Gyöngyi, Székely Gábor, Ágoston Péter, Jorgó Kliton, Polgár Csaba, Jurányi Zsolt. Biodozimetriai értékek és különböző módszerekkel számolt sugárterápiás térfogatok összevetése három féle kezelés esetén. 2018. május 10-12, Székesfehérvár.

Farkas Gyöngyi, Kocsis S. Zsuzsa, Székely Gábor, Mihály Dalma, Pesznyák Csilla, Major Tibor, Polgár Csaba, Jurányi Zsolt A citogenetika szerepe a sugárvédelemben XLIII. Sugárvédelmi továbbképző tanfolyam, Hajdúszoboszló.

Farkas Gyöngyi, Környezet, genetika és rák. Betegoktatási program, Országos Onkológiai Intézet, 2018.

Jurányi Zsolt. Teendők rákterápia alatt – a betegek szemszögéből. Betegoktatási program, Országos Onkológiai Intézet, 2018.

Farkas Gyöngyi, Környezet, genetika és rák, Kutatók éjszakája, 2018, Országos Onkológiai Intézet.

Jurányi Zsolt. Daganatos betegségek Magyarországon és világszerte: okok, gyakoriság, megelőzés Kutatók éjszakája, 2018, Országos Onkológiai Intézet.

Kocsis S. Zsuzsa. Betekintés a tumorbiológiába és a terápiák biológiájába. Kutatók éjszakája, 2018, Országos Onkológiai Intézet.

2019

Zsuzsa S. Kocsis, Péter Ágoston, Gyöngyi Farkas, Márta Kun-Gazda, Gábor Székely, Tibor Major, Dalma Mihály, Csilla Pesznyák, Gábor Stelczer, Kliton Jorgo, László Gesztesi, Csaba Polgár, Zsolt Jurányi.
Comparison of chromosome aberrations and side effects in four modalities of prostate cancer radiotherapy, International Congress of Radiation Research, 2019, augusztus 25-29, Manchester.

MST kongresszus, Lilafüred, 2019.05.16.-05.18.

Kromoszómaaberrációk és mellékhatások vizsgálata négyféle prosztata sugárkezelés után
Kocsis S. Zsuzsa, Ágoston Péter, Farkas Gyöngyi, Kun-Gazda Márta, Székely Gábor, Major Tibor, Mihály Dalma, Pesznyák Csilla, Stelczer Gábor, Jorgo Kliton, Gesztesi László, Polgár Csaba, Jurányi Zsolt

Tüdőtumoros betegek sztereotaxiás ablatív sugárkezelése után a légzésfunkció változás és a kromoszómaaberrációk kapcsolata
Farkas Gyöngyi, Bajcsay András, Ostoros Gyula, Markóczy Zsolt, Kocsis S. Zsuzsa, Kun-Gazda Márta, Budai Mariann, Székely Gábor, Mihály Dalma, Lövey József, Polgár Csaba, Jurányi Zsolt

Sugárkezelés és immunterápia: az új nyerő kombináció?
Jurányi Zsolt, Kocsis S. Zsuzsa, Lumniczky Katalin, Balázs Katalin, Farkas Gyöngyi, Kun-Gazda Márta, Székely Gábor, Polgár Csaba

Sugárrezisztens HCC1954 emlőtumor sejtvonal létrehozása és jellemzése
Kun-Gazda Márta, Kocsis S. Zsuzsa, Herein András, Polgár Csaba, Nagy Péter, Jurányi Zsolt

MOT kongresszus, (2019. november 28-30)
HDR, seed brachyterápia, LINAC és Cyberknife alapú prosztata sugárkezelés összehasonlítása biodozimetriai és klinikai szempontból
Kocsis S. Zsuzsa, Ágoston Péter, Farkas Gyöngyi, Kun-Gazda Márta, Székely Gábor, Major Tibor, Mihály Dalma, Pesznyák Csilla, Stelczer Gábor, Jorgo Kliton, Gesztesi László, Polgár Csaba, Jurányi Zsolt

Sugárrezisztens HCC1954 emlőtumor sejtvonal létrehozása frakcionált besugárzással
Kun-Gazda Márta, Kocsis S. Zsuzsa, Herein András, Polgár Csaba, Nagy Péter, Jurányi Zsolt,

Nem kissejtes tüdőtumoros betegek sztereotaxiás ablatív sugárkezelését követően a légzésfunkciós paraméterek és a kromoszómaaberrációk vizsgálata
Farkas Gyöngyi, Bajcsay András, Ostoros Gyula, Markóczy Zsolt, Kocsis S. Zsuzsa, Kun-Gazda Márta, Budai Mariann, Székely Gábor, Mihály Dalma, Lövey József, Polgár Csaba, Jurányi Zsolt

ICRR, Manchester, 2019, 08.25-29.
Comparison of chromosome aberrations and side effects in four types of prostate cancer radiotherapy
Zsuzsa S. Kocsis, Péter Ágoston, Gyöngyi Farkas, Márta Kun-Gazda, Gábor Székely, Tibor Major, Dalma Mihály, Csilla Pesznyák, Gábor Stelczer, Kliton Jorgo, László Gesztesi, Csaba Polgár , Zsolt Jurányi

Farkas Gyöngyi, Környezet, genetika és rák. Betegoktatási program, Országos Onkológiai Intézet, 2019.

Jurányi Zsolt. A sugárterápia hatásmechanizmusa. Betegoktatási program, Országos Onkológiai Intézet, 2019.

Farkas Gyöngyi, Környezet, genetika és rák, Kutatók éjszakája, 2019, Országos Onkológiai Intézet.

Jurányi Zsolt. Daganatos betegségek Magyarországon és világszerte: okok, gyakoriság, megelőzés Kutatók éjszakája, 2019, Országos Onkológiai Intézet.

Jurányi Zsolt. Biológiai dozimetria. Klinikai sugárbiológia tanfolyam, 2019.10.10, Budapest.

Kocsis S. Zsuzsa. Betekintés a tumorbiológiába és a terápiák biológiájába. Kutatók éjszakája, 2019, Országos Onkológiai Intézet.

2020
ESTRO kongresszus, Bécs, online poszter 2020. 07.31-08.04.
Cytogenetic and immunological markers to predict side effects and tumour control in cancer patients. Zsolt Juranyi, Zsuzsa Kocsis S., Katalin Lumniczky, Katalin Balázs, Péter Ágoston, Gyöngyi Farkas, Márta Kun-Gazda, Gábor Székely, Tibor Major, Csilla Pesznyák, Gábor Stelczer, Kliton Jorgo, László Gesztesi, Csaba Polgár, Géza Sáfrány.

MST szimpózium 2020.10.16-17.
A sugárkezelés és az immunterápia kombinációja.
Mikor, hogyan előnyök, hátrányok, Jurányi Zsolt, Kocsis S. Zsuzsa, Lumniczky Katalin, Balázs Katalin, Farkas Gyöngyi, Kun-Gazda Márta, Székely Gábor, Polgár Csaba

Pécsi Immuno-Onkológiai Napok, 2020.02.07-08.
A tumor kontroll és a radiogén mellékhatások prediktív citogenetikai és immunológiai biomarkerei négyféle sugárterápiás modalitással kezelt prosztata rákos betegeknél, Jurányi Zsolt, Kocsis S. Zsuzsa, Lumniczky Katalin, Balázs Katalin, Ágoston Péter, Farkas Gyöngyi, Kun-Gazda Márta, Székely Gábor, Major Tibor, Pesznyák Csilla, Stelczer Gábor, Jorgo Kliton, Gesztesi László, Polgár Csaba, Sáfrány Géza

2021

ESTRO kongresszus, Madrid, online poszter, 2021 Augusztus 27-31.
Cytogenetic and immunological biomarkers with predictive value for the development of side effects and tumour control in prostate cancer patients treated with four types of radiotherapy
Zsolt Jurányi, Zsuzsa S. Kocsis, Katalin Lumniczky, Katalin Balázs, Péter Ágoston, Gyöngyi Farkas, Viktória Tölgyesi, Gábor Székely, Tibor Major, Csilla Pesznyák, Gábor Stelczer, Kliton Jorgo, László Gesztesi, Géza Sáfrány, Csaba Polgár.

MOT 34. kongresszus, Szeged, 2021. november 11-13.
Sugárbiológiai módszerek a sugárterápiás mellékhatások predikciójára, Kocsis S. Zsuzsa, Ágoston Péter, Farkas Gyöngyi, Kun-Gazda Márta, Székely Gábor, Major Tibor, Mihály Dalma, Pesznyák Csilla, Stelczer Gábor, Jorgo Kliton, Gesztesi László, Polgár Csaba, Jurányi Zsolt.

MST 15. kongresszus, HOTEL CLUB TIHANY, 2021. szeptember 23-25.
Kromoszómaaberrációk és sugárterápiás mellékhatások kapcsolata: négyféle prosztata sugárterápia összehasonlítása biodozimetriai szempontból, Kocsis S. Zsuzsa, Ágoston Péter, Farkas Gyöngyi, Kun-Gazda Márta, Székely Gábor, Major Tibor, Mihály Dalma, Pesznyák Csilla, Stelczer Gábor, Jorgo Kliton, Gesztesi László, Polgár Csaba, Jurányi Zsolt.
MST 15. kongresszus, HOTEL CLUB TIHANY, 2021. szeptember 23-25.
Radiation Induced Lymphocyte Apoptosis (RILA) és kromoszómaaberrációs módszer összehasonlítása a sugárterápiás mellékhatások előrejelzésének szempontjából, Jurányi Zsolt, Kocsis S. Zsuzsa, Ágoston Péter, Farkas Gyöngyi, Kun-Gazda Márta, Székely Gábor, Major Tibor, Mihály Dalma, Pesznyák Csilla, Stelczer Gábor, Jorgo Kliton, Gesztesi László, Polgár Csaba, Jurányi Zsolt.

MST 15. kongresszus, HOTEL CLUB TIHANY, 2021. szeptember 23-25.
A sugárterápiában alkalmazott különféle fotonsugárzási paraméterek biológiai hatásának összehasonlítása biológiai dozimetriával, Farkas Gyöngyi, Székely Gábor, Kocsis S. Zsuzsa, Pócza Tamás, Mihály Dalma, Herein András, Pesznyák Csilla, Major Tibor, Polgár Csaba, Jurányi Zsolt.

Betegoktatás: Farkas Gyöngyi: Környezet, genetika és rák, 2021.

Jurányi Zsolt. Biológiai dozimetria. Klinikai sugárbiológia tanfolyam, 2021.10.06, Budapest.

2022

ESTRO kongresszus, Koppenhága, 2022. 05.06-05.10.
Predictive biomarkers for side effects and tumor control in radiotherapy-treated prostate cancer patients, Zsolt Jurányi, Zsuzsa S. Kocsis, Katalin Lumniczky, Katalin Balázs, Péter Ágoston, Gyöngyi Farkas, Viktória Tölgyesi, Gábor Székely, Tibor Major, Csilla Pesznyák, Gábor Stelczer, Kliton Jorgo, László Gesztesi, Csaba Polgár, Géza Sáfrány

Participation at scientific meetings between 2017-2022 (NPHC):

2017

18-20.10.2017.: Hungarian Society of Immunology, poster: Radiotherapy-induced phenotypical changes in peripheral blood mononuclear cells of head and neck cancer patients, Balázs Katalin, Kis Enikő, Christophe Badie, Bogdándi Noémi Enikő, Serge Candéias, Lourdes Cruz Garcia, Iwona Dominczky, Benjamin Frey, Udo Gaipl, Jurányi Zsolt, Kocsis S. Zsuzsa, Eric Andreas Rutten, Sáfrány Géza, Piotr Widlak, Lumniczky Katalin

16-18.11.2017.: Hungarian Cancer Society, poster: Sugárterápia indukált fenotipikus változások fej-nyaki daganatos betegek perifériás mononukleáris sejtjeiben, Balázs Katalin, Kis Enikő, Christophe Badie, Bogdándi Noémi Enikő, Serge Candéias, Lourdes Cruz Garcia, Iwona Dominczky, Benjamin Frey, Udo Gaipl, Jurányi Zsolt, Kocsis S. Zsuzsa, Eric Andreas Rutten, Sáfrány Géza, Piotr Widlak, Lumniczky Katalin

2018

21-25.08.2018.: European Radiation Research Society (**ERRS**), poster: Radiotherapy-induced cellular and soluble immunological markers of head and neck cancer patients, (**Young Investigator Award** was obtained), Balázs Katalin, Kis Enikő, Christophe Badie, Bogdándi Noémi Enikő, Serge Candéias, Lourdes Cruz Garcia, Iwona Dominczky, Benjamin Frey, Udo Gaipl, Jurányi Zsolt, Kocsis S. Zsuzsa, Eric Andreas Rutten, Sáfrány Géza, Piotr Widlak, Lumniczky Katalin

29-31.08.2018.: Congress of Hungarian Association of Public Health Training and Research Institute (**HAPHI**), oral presentation: Sugárzás indukálta celluláris és szolubilis immunológiai markerek vizsgálata fej-nyaki daganatos betegekben, Balázs Katalin, Kis Enikő, Christophe Badie, Bogdándi Noémi Enikő, Serge Candéias, Lourdes Cruz Garcia, Iwona Dominczky, Benjamin Frey, Udo Gaipl, Jurányi Zsolt, Kocsis S. Zsuzsa, Eric Andreas Rutten, Sáfrány Géza, Piotr Widlak, Lumniczky Katalin

14.09.2018.: Congress of Young Oncologist, oral presentation: Fej- nyaki daganatos betegek sugárterápia-specifikus immunológiai markereinek vizsgálata, Balázs Katalin, Kis Enikő, Christophe Badie, Bogdándi Noémi Enikő, Serge Candéias, Lourdes Cruz Garcia, Iwona Dominczky, Benjamin Frey, Udo Gaipl, Jurányi Zsolt, Kocsis S. Zsuzsa, Eric Andreas Rutten, Sáfrány Géza, Piotr Widlak, Lumniczky Katalin

26.10.2018.: I. Flow Cytometry Day - Semmelweis University, Doctoral School of Pathological Sciences, poster: Celluláris és szolubilis biomarkerek vizsgálata sugárterápiával kezelt fej-nyaki daganatos betegek vérében**,** Balázs Katalin, Kis Enikő, Christophe Badie, Bogdándi Noémi Enikő, Serge Candéias, Lourdes Cruz Garcia, Iwona Dominczky, Benjamin Frey, Udo Gaipl, Jurányi Zsolt, Kocsis S. Zsuzsa, Eric Andreas Rutten, Sáfrány Géza, Piotr Widlak, Lumniczky Katalin

08-10.11.2018.: Congress of Hungarian Society of Clinical Oncology, oral presentation: Sugárterápia-specifikus immunológiai markerek vizsgálata fej-nyaki daganatos betegek, Balázs Katalin, Kis Enikő, Christophe Badie, Bogdándi Noémi Enikő, Serge Candéias, Lourdes Cruz Garcia, Iwona Dominczky, Benjamin Frey, Udo Gaipl, Jurányi Zsolt, Kocsis S. Zsuzsa, Eric Andreas Rutten, Sáfrány Géza, Piotr Widlak, Lumniczky Katalin

2019

25-29.08.2019.: 16th International Congress of Radiation Research (**ICRR**, Manchester), poster and oral presentation: The effect of radiotherapy on the immune phenotype of prostate

cancer patients, Balázs Katalin, Kocsis S. Zsuzsa, Ágoston Péter, Jorgo Kliton, Gesztesi László, Farkas Gyöngyi, Székely Gábor, Takácsi-Nagy Zoltán, Polgár Csaba, Sáfrány Géza, Jurányi Zsolt, Lumniczky Katalin

16-18.05.2019.: Congress of Hungarian Society of Radiotherapy, poster: Celluláris és szolubilis biomarkerek vizsgálata sugárterápiával kezelt fej-nyaki daganatos betegekben, Balázs Katalin, Kocsis S. Zsuzsa, Ágoston Péter, Jorgo Kliton, Gesztesi László, Farkas Gyöngyi, Székely Gábor, Takácsi-Nagy Zoltán, Polgár Csaba, Sáfrány Géza, Jurányi Zsolt, Lumniczky Katalin

28-30.11.2019.11.: Hungarian Cancer Society, poster: Brachi – és teleterápia hatása prosztata adenokarcinómás betegek immunfenotípusára, Balázs Katalin, Kocsis S. Zsuzsa, Ágoston Péter, Jorgo Kliton, Gesztesi László, Farkas Gyöngyi, Székely Gábor, Takácsi-Nagy Zoltán, Polgár Csaba, Sáfrány Géza, Jurányi Zsolt, Lumniczky Katalin

2020

07-08.02.2020.: Congress of Immuno-Oncology, Pécs (**PION**), oral presentation: Immunológiai biomarkerek prosztatarák sugárkezelésénél, Balázs Katalin, Kocsis S. Zsuzsa, Ágoston Péter, Jorgo Kliton, Gesztesi László, Farkas Gyöngyi, Székely Gábor, Takácsi-Nagy Zoltán, Polgár Csaba, Sáfrány Géza, Jurányi Zsolt, Lumniczky Katalin

2021

05-07.11.2021: Hungarian Molecular Life Science Congress: poster: Radiotherapy-induced changes in systemic immune parameters of prostate cancer patients, Balázs Katalin, Kocsis S. Zsuzsa, Ágoston Péter, Jorgo Kliton, Gesztesi László, Farkas Gyöngyi, Székely Gábor, Takácsi-Nagy Zoltán, Polgár Csaba, Sáfrány Géza, Jurányi Zsolt, Lumniczky Katalin

09-12.06.2021: Virtual congress of European Association for Cancer Research (**EACR**), poster presentation: The influence of low- and high-dose rate brachytherapy on systemic immunological markers in prostate cancer patients, Balázs Katalin, Kocsis S. Zsuzsa, Ágoston Péter, Jorgo Kliton, Gesztesi László, Farkas Gyöngyi, Székely Gábor, Takácsi-Nagy Zoltán, Polgár Csaba, Sáfrány Géza, Jurányi Zsolt, Lumniczky Katalin

11-13.11.2021: Congress of Hungarian Cancer Society (Magyar Onkológusok Társasága-**MOT**), poster: Az alacsony és magas dózisteljesítményű brachyterápia hatása prosztata daganatos betegek szisztémás immunológiai paramétereire, Balázs Katalin, Kocsis S. Zsuzsa, Ágoston Péter, Jorgo Kliton, Gesztesi László, Farkas Gyöngyi, Székely Gábor, Takácsi-Nagy Zoltán, Polgár Csaba, Sáfrány Géza, Jurányi Zsolt, Lumniczky Katalin

22-22.11.2021: Congress of European Radiation Protection Week (**ERPW**), oral presentation: The effect of low- and high-dose rate brachytherapy on the immune phenotype of prostate cancer patients, Balázs Katalin, Kocsis S. Zsuzsa, Ágoston Péter, Jorgo Kliton, Gesztesi László, Farkas Gyöngyi, Székely Gábor, Takácsi-Nagy Zoltán, Polgár Csaba, Sáfrány Géza, Jurányi Zsolt, Lumniczky Katalin

2022

21-24.09.2022.: European Radiation Research Society (**ERRS**), poster: The effect of low-and high-dose rate brachytherapy on the innate and adaptive immune system of prostate cancer patients, Balázs Katalin, Kocsis S. Zsuzsa, Ágoston Péter, Jorgo Kliton, Gesztesi László, Farkas

Gyöngyi, Székely Gábor, Takácsi-Nagy Zoltán, Polgár Csaba, Sáfrány Géza, Jurányi Zsolt, Lumniczky Katalin

Summary of performed research

The goal of this collaborative project between the National Institute of Oncology (NIO) and National Public Health Centre (NPHC) is to investigate how cancer and radiotherapy as a major anti-cancer treatment modality impact the immune system long after the successful treatment of a tumor and to follow-up systemic side effects of different types of radiotherapy up to 5 years after therapy.

Prostate cancer patients were treated with different radiotherapy modalities. During LINAC-based teletherapy total dose is delivered in several fractions. Its state-of-the-art form is Cyberknife therapy with high precision robotics and narrow beams. Brachytherapy techniques use radioisotopes placed inside the body: near or into the tumor. LDR (low dose rate) brachytherapy is performed by permanent insertion of isotopes. HDR (high dose rate) brachytherapy also uses isotopes, but after quick dose transmission they are removed from the patient. Prostate cancer can be treated with all the aforementioned techniques, therefore, different radiobiological effects are comparable in this patient population. Doses: HDR brachyterapy 1 x 19/21 Gy, LDR brachytherapy 1 x 145 Gy over 12 months, teletherapy (LINAC-based) delivered in multiple 1,8-2,5 Gy fractions up to 70-78 Gy total dose, Cyberknife 5 x 8 Gy.

We recruited 251 patients and had to exclude 8 individuals. The patients were treated with seed brachytherapy (69 patients), high dose rate (HDR) brachytherapy (52 patients), conventional teletherapy (55 patients) and cyberknife teletherapy (65 patients) out of which approx. 20 patients in the first three groups were investigated for systemic immune changes as well. There was one deviation from the protocol. We omitted combined radiotherapy patients (only recruited two), because we realised, the combination of conventional teletherapy and two fractions of HDR brachytherapy can be administered in three schedules causing too many small subgroups. We recruited patients undergoing cyberknife robotic teletherapy instead. This treatment was not available in our centre at the time of the contract. The new accelerator arrived as first of the kind in Central Europe, and we started to recruit these patients in May of 2018. There were also more patients included in the study than in the contract. The required ethical permissions were modified accordingly.

Changes in cytogenetic and immune parameters were investigated before, right after and 3, 6, 9, 12, 24, 36 months after therapy and samples are collected annually for further time points as well.

Clinical data were recorded using RTOG/EORTC genitourinary (GU) and gastrointestinal (GI) grade, IPSS (International Prostate Symptom Score) and QoL (Quality of life) questionnaires. Blood samples were collected up to the present at National Institute of Oncology (NIO) and transferred to National Public Health Centre (NPHC) for further analyses.

National Institute of Oncology (NIO)

In our study population, there were 23.7% low, 61.8% intermediate and 14.5% high risk prostate cancer patients. The survival of the non-metastatic prostate cancer is usually high, even if they are an older cohort among the cancer patients. The average age was 67.7 years in our cohort. The overall survival was between 97.4% (cyberknife group) and 89.9% (teletherapy group). The success of radiotherapy can be best described by the local relapse free survival, which was 98.1 ± 1.9%, 100%, 96.7 ± 2.3% and 100% in the cyberknife, HDR, seed, and conventional teletherapy group, respectively. The biochemical relapse is defined by the prostate

specific antigen (PSA) level increase in the blood (more than 2 ng/ml increase compared of the nadir level). The increase of this marker is one of the earliest signs of relapse, (however, not entirely specific) and gives opportunity to intervene. We observed at least 82.7 ± 5.8% biochemical relapse free survival (HDR brachytherapy group). The maximum was 93.8 ± 3.0% in the seed brachytherapy group.

We registered the side effects of the patients at the same time points when blood samples were taken. There was a control visit at the physician directly after the last fraction of the radiation, and after 3, 6, 9, 12, 24, 36, 48, 60 months. These four radiotherapy modalities have not been ever compared in the same cohort. We found that the HDR therapy caused the less early urogenital toxicities, while the other three modalities showed similar effects. There was few (1.6-2.9%) severe, grade 3 side effects. Among the acute gastrointestinal toxicities we did not find any of these severe side effects. The conventional teletherapy caused the most grade 2 (manageable with medication) early side effects (14.8%). The late urinary side effects were the least frequent in case of HDR therapy and cyberknife teletherapy. However, grade 3 severe side effects emerged in similar frequencies in all treatment groups (2.4-3.3%). There were few and mild late gastrointestinal side effects. There was no grade 3 toxicity and most patients (69.0-94.7%) have not suffered any intestinal symptom.

The questionnaires about the side effects filled by the patients were also be recorded. We used the International Prostate Syndrome Score (IPSS) assessing urinary problems and Quality of Life (QoL) internationally accepted questionnaires (by RTOG-EORTC). The patients reported the most severe side effects directly after the completion of the two kind of teletherapies in both questionnaires. The average IPSS score was 16.7 ± 1.2 (the most severe toxicity score would be 35) and 16.3 ± 1.4 in the cyberknife and conventional teletherapy group, respectively. On the other hand, it is meaningless to assess side effects one day after brachytherapy (as we would measure the effect of invasive intervention). The highest side effect score was reported at three months in case of brachytherapies. The IPSS score at that time was 14.8 ± 0.8 for seed brachytherapy and only 7.1 ± 0.7 for the patient group treated with HDR isotope therapy. The QoL score showed similar decrease after three months in all groups. The worst average score was 3.1 ± 0.3 (the range is 0-5) observed directly after conventional teletherapy.

The other purpose of this study was observing chromosome aberrations (CAs) in the different radiotherapy groups to compare the biological dose. As the physical dose is modified by the sensitivity of different tissues and individual radiosensitivity, the biological dose should also be analysed to assess the effects of radiotherapy. The CA technique is the gold standard of biological dosimetry, which measures the absorbed dose and the individual sensitivity together.

We found that the chromosome aberrations were the highest near three months after the radiotherapy: directly after therapy for HDR, at three months for conventional teletherapy and at six months for cyberknife therapy and 12 months for seed brachytherapy. The values showed high variance between therapies. The teletherapy regimens caused significantly more aberrations than the brachytherapy technics. For example, the maximum total aberration value (/100 cells) was 17.94 ± 1.9 for conventional teletherapy; 13.98 ± 2.4 for cyberknife; 7.2 ± 0.5 for seed and 6.1 ± 1.0 for HDR brachytherapy. For comparison, 5 aberration /100 cells is considered normal for a healthy individual. After 12 months the chromosome aberrations decreased but in case of conventional teletherapy and seed brachytherapy it did not reach the starting value or the 5 aberration /100 cells baseline. The singular chromosome aberrations: chromatid and chromosome breaks, exchanges, translocations, dicentrics and rings followed the same curse of increase and decrease. The dicentrics and ring chromosomes are radiation specific aberrations. There is no baseline for dicentrics + rings (although 1/1000 cells is published to be the occurance in healthy persons), but they also did not decrease back to the starting value.

The conclusion of our study is that radiotherapy regimens with similar efficiency can cause very different biological dose on the immune system. The impact of these differences can be in the long-term efficiency, in the development of side effects or secondary cancers (later not in prostate cancer patients). On the other hand, we firstly showed, that the damage on lymphocytes was not completely eliminated till five years. The explanation can be, that damaged lymphocytes - which can have long lifespan - can be stored in the lymph nodes and getting back to the circulation years later. These findings can be important in the light of emergence of immuntherapies. The lymphocytes, which are induced to kill tumor cells are already damaged and not restored completely after certain radiotherapy regimens. The aberrant cell values show us, that there can be 13% damaged T-lymphocyte three months after conventional teletherapy.

We searched for connections between the chromosomal aberrations and side effects in order to decide if CAs can predict toxicities. One of the most difficult factor of radiotherapy to deal with is individual radiosensitivity. Approximately 5-10% of the patients develop severe symptoms due to side effects although they get the same dose as other patients. If these patients could be assigned before therapy, alternative therapy, another modality (brachytherapy as our study suggests) or altered dose could be given. This way, a lot of inconvenience for the patients and cost for the healthcare system could be spared. Biomarker for this purpose has been searched since 1960's but there is not any in clinical use.

We could show correlations between chromosomal aberrations and side effects measured at the same time points. For example, six months after seed therapy chromatid breaks, total aberrations and aberrant cell number correlated with IPSS and QoL score (Spearman correlation coefficient (r=0.25-0.31). Six months after HDR brachytherapy chromatid breaks, total aberrations and aberrant cell number correlated with IPSS and QoL (r=0.31-0.44). A year after teletherapy total aberrations correlated with IPSS (r=0.28).

Furthermore, we found that CAs shortly after radiotherapy correlated with cumulative late side effects, predicting them: Directly after seed brachytherapy total aberrations, aberrant cell frequency and chromatid breaks correlated with late genitourinary side effects (r=0.25, 0.26 and 0.34, respectively). Six months after seed therapy chromatid breaks also correlated with cumulative GI toxicities (r=0.31).

In summary we showed the power of chromosome aberration technique predicting side effects and possibly used as a biomarker to modify tumor therapy in case of radiation sensitive patients. Based on our study, informed patients and physicians may choose better between radiotherapy techniques, which could contribute to their wellbeing.

National Public Health Center (NPHC)

Further analyses of peripheral blood mononuclear cells (PBMCs) and plasma samples of prostate cancer patients were performed at NPHC. Based on the first results of LDR patients we further analysed the promising immune cell subsets with extended immunophenotypic panels in HDR and teletherapy groups which enabled us to study a wide range of immune cells with much more functional details as compared to what we described at the beginning of this project.

To better understand the background of prostate cancer development and the currently investigated cancer specific molecular biomarkers a scientific publication about immune-related predictive and prognostic markers in radiotherapy-treated patients was published entitled: Blood-derived biomarkers of diagnosis, prognosis and therapy response in prostate cancer patients (https://doi.org/10.3390/jpm11040296).

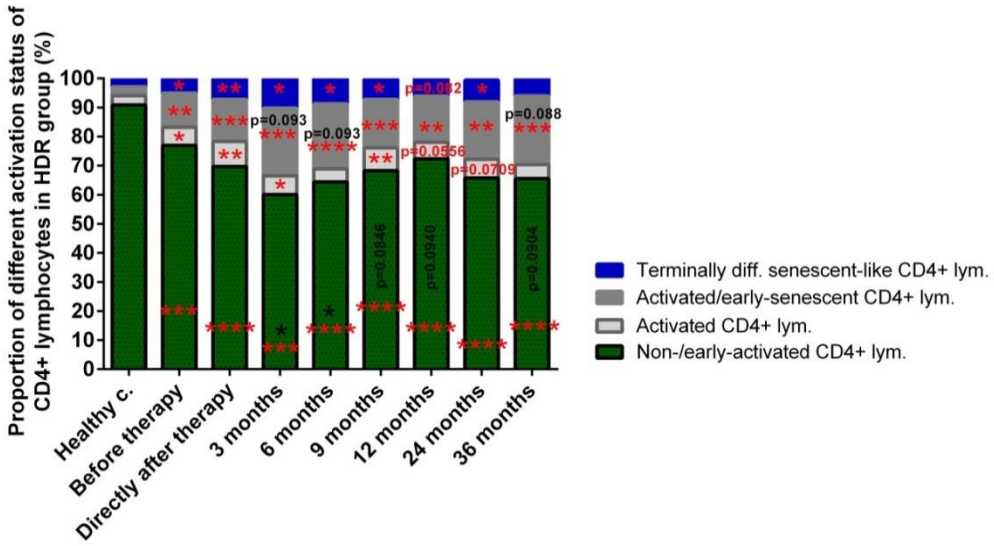
At NPHC we finalized immune phenotyping of PBMCs and the protein profile investigation of 21 **LDR** patients having blood samples collected up to 36 months and performed statistical analysis of cellular, soluble, clinical parameters. PBMC samples of 36 age-matched healthy individuals were analysed as healthy control group. We investigated the amount of effector and regulatory T cells (Tregs) and the expression level of their important functional cell surface markers (CTLA-4, PD-1, CD39, Ki-67, FoxP3), as well as natural killer (NK) cells and dendritic cells (DCs), , which enabled us to study both innate and adaptive immune responses of prostate cancer patients after radiotherapy. The amount of CTLA-4+ regulatory T cells did not change significantly during the 36 months follow-up compared to control group, however the distribution of FoxP3+ and FoxP3- cells within the CTLA-4+ CD4+ lymphocytes showed that the fraction of FoxP3+ cells was almost 2-fold higher in patients before seed implantation compared to controls and this shift towards FoxP3+ cells continued to increase during the 36 months follow-up. Moreover, the median fluorescence intensity (MFI) of CTLA-4 expression on FoxP3+CD4+ Tregs progressively increased in cancer patients after the initiation of radiotherapy, reaching significantly higher levels 24 and 36 months after implantation compared to both healthy controls and cancer patients before treatment. The level of PDGF-AA, ENA-78 and RANTES increased in the plasma of patients before therapy and normalized at later time points. These results of LDR patients were published: Prostate Cancer Survivors Present Long-Term, Residual Systemic Immune Alterations (https://doi.org/10.3390/cancers14133058). The main conclusions of this study are that in LDR patients before therapy both the adaptive (decreased CD4+ T lymphocytes, increased Ki-67+ proliferated CD4+ T cells) and the innate immune system (decreased NK cell fraction and mature NK cells, increased anergic NK cells and lymphoid DCs) were altered compared to healthy control group. The innate immune response recovered as the tumour was cured, nevertheless a mild long-term deficit in the adaptive immune response persisted even 3 years later (decreased CD4+ T lymphocytes and strongly increased FoxP3+ regulatory T cells).

Phenotypical changes of PBMCs from 22 patients receiving **HDR** brachytherapy were investigated in a similar manner to LDR brachytherapy patients, nevertheless with enlarged phenotypical panels which allowed identification of more functional subgroups within the different lymphocyte subpopulations without compromising the comparability of the data with LDR patients. The major changes compared to the LDR patient group was that we included B cells, exhausted and senescence CD4+ and CD8+ T cells and different subtypes of memory CD4+ and CD8+ T cells in our analysis. PBMC samples of 22 age-matched healthy individuals were analysed as healthy control group.

In healthy controls the majority of CD4+ cells were non-activated, naive cells (representing 90%), while in the case of CD8+ cells the distribution between naïve/activated CD8+ cells and CD8+ cells in various stages of senescence was approx. 50-50%. An important finding was that the proportion of non/early/activated/senescent T cells was different in case of CD4+ and CD8+ T cells in patients before treatment compared to healthy controls. In cancer patients, the fraction of CD4+ non/early activated T cells significantly decreased compared to control group up and this change persisted up to 36 months not being influenced by therapy or tumour cure. The fraction of activated CD4, activated/early senescent as well as terminally differentiated CD4+ cells increased in cancer patients before therapy compared to control. The fraction of the latter two population progressively further increased during the 36 months follow-up, nevertheless changes were not statistically significant. The activation status of the CD8+ cells in cancer patients before treatment was also changed compared to healthy controls leading to a decrease in the fraction of non/early activated states and an increase in the activated/early senescence state. This altered distribution pattern was maintained throughout the follow-up period (Figure 1A, B). These findings indicate a deficit in the maturation capacity of both CD4+ and CD8+ cells of prostate cancer patients. The fact that the deficit persisted long

time after treatment and that it was not influenced by therapy might be an indication that the detected functional deficits were intrinsically present in patients and might have contributed to the development of the malignant state.

A)



B)

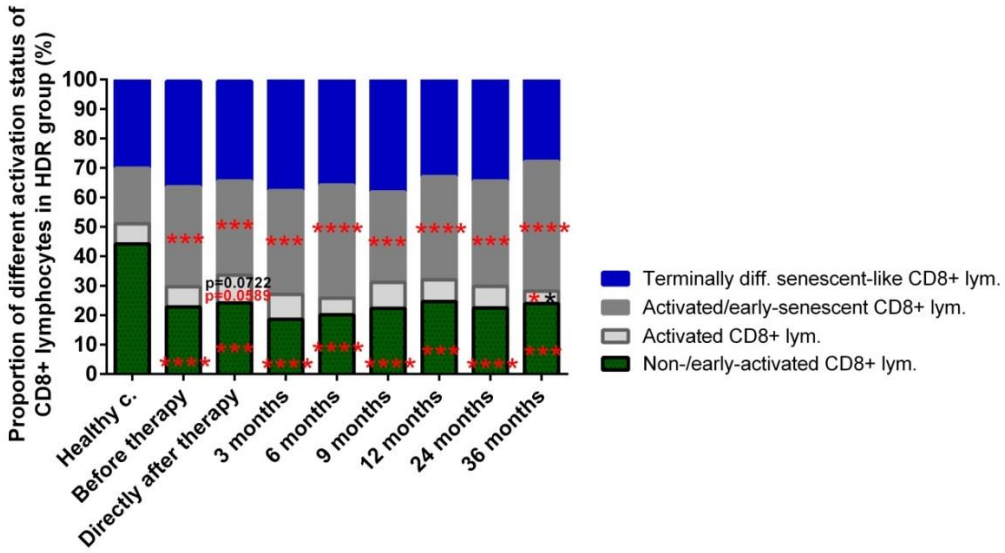


Figure 1. Changes in the fraction of the four different activation status of CD4+ (A) and CD8+ (B) T cells in healthy controls and in prostate cancer patients treated with HDR. Red asterisks represent significant changes of cancer patients compared to controls. Black asterisks represent significant changes of patients after treatment compared to pre-treatment values.

Radiotherapy-induced changes in the different subtypes of memory cells were very similar in both CD4+ and CD8+ T cells. Central and terminal memory T cell levels were below control during the whole follow-up up to 36 months.

Based on TNM, Gleason grade of the tumours and plasma PSA level patients were grouped in different risk classes as mentioned in the report by NIO as well. HDR patients could be categorized only in low and medium risk patients. We found interesting associations of various immune phenotypes with these risk categories (Table 1): the level of activated CD8+ T cells

and anergic NK cells were significantly lower in medium risk group compared to low risk group, while the fraction of NKT-like cells was significantly higher in medium risk group compared to low risk patients group.

	Risk group 1	Risk group 2		Risk group 1	Risk group 2
Immature precursor NK cells	↑	↓	Mature NK cells	↓	↑
Immature/early mature NK cells	↑	↓	NK cells (CD16+CD56+ in CD3- lym)	↓	↑
Anergic NK cells	↑ *	↓ *	NKT-like cells	↓ *	↑ *
Lymphoid DC	↑	↓	B cells	↓	↑
CD3+CD4+ lymphocytes	↑	↓	Effector T lymphocytes	↓	↑
Activated CD4+	↑	↓	Non/early activated CD4+ lym	↓	↑
Activated CD8+	↑ *	↓ *	Non/early activated CD8+ lym	↓	↑
CCR4+ Tregs	↑	↓	Regulatory T cells	↓	↑
Activated/early senescent CD4+	↑	↓	CD44+ Tregs	↓	↑
			CTLA4+ Tregs	↓	↑

Table 1. Summary of the changes of various immune phenotypes between the low (risk=1) and medium (risk=2) risk groups in cancer patients receiving HDR therapy. Black asterisks represent significant changes between the risk groups and the arrows show the direction of changes.

We further analysed the relationship between persistent chromosome aberrations (reported by NIO) and chronic, long-lasting changes in the immunophenotype of peripheral lymphocytes in HDR group. We found correlations between the level of total aberrations directly after HDR brachytherapy and the level of CD4+ terminally differentiated senescent cells in patients who had chromosome aberrations over the cut-off limit (5 aberrations/100 cells) after the therapy, possibly indicating a direct functional deficit of these cells with persistent chromosomal damage.

The level of PDGF-AA, ENA-78, RANTES and VEGF increased in the plasma of patients before therapy, which tended to normalize at later time points after therapy. However, PDGF did not normalize in a faction of patients. Persistently increased PDGF-AA levels might be an indication of an increased risk of radiation-induced late fibrosis, therefore these patients are being followed up.

A manuscript has been prepared summarizing our immunological data on HDR patients, which is currently finalized and it is expected to be submitted in a radiation oncology journal in the next 1-2 months. The preliminary title of the manuscript is: Acute and long-lasting immunological changes in prostate cancer patients treated with high-dose rate brachytherapy.

The phenotype of PBMCs from 20 patients receiving **LINAC-based teletherapy** were investigated with the same phenotyping panels as in HDR group complemented with 2 newly designed panels allowing a more detailed analysis of B cells (transitional, naïve mature, activated, early and late memory B cells and antigen-presenting plasma cells), monocytes/macrophages (non-classical, intermediate, classical monocytes). In addition, based on recent literature data [1] reporting that prostate-specific antigen (PSA) can be detected in macrophages and PSA-containing macrophages might be biomarkers of prostate cancer we investigated the fraction of PSA+ macrophages and compared them with blood PSA levels.

The amount of PSA-containing macrophages increased 3-fold in cancer patients before therapy compared to control group and we found positive correlation (r=0.554) between the plasma PSA level and the level of circulating PSA+ macrophages (Figure 2) indicating that PSA+ macrophages are also useful markers of prostate cancer.

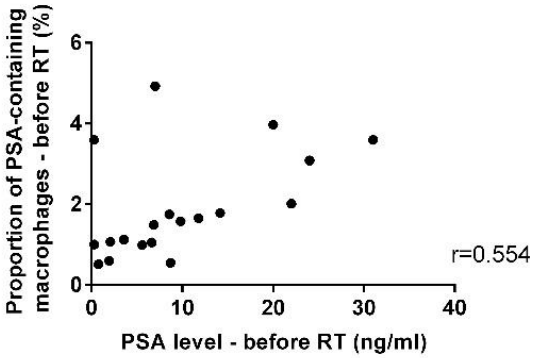
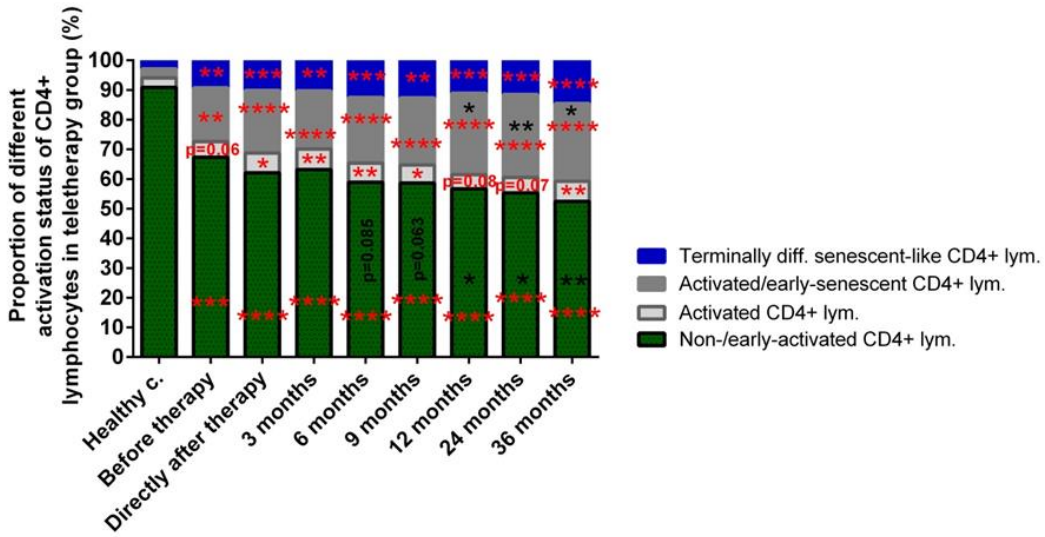


Figure 2: Correlation between plasma PSA levels and PSA-positive circulating macrophages in prostate cancer patients before therapy.

The proportion of non-senescent and senescent CD4+ and CD8+ T cells changed very similarly to HDR group, however terminally differentiated CD4+ and CD8+ cells showed much stronger elevation in teletherapy group compared to controls than in the HDR group (Figure 3A, B).

A)



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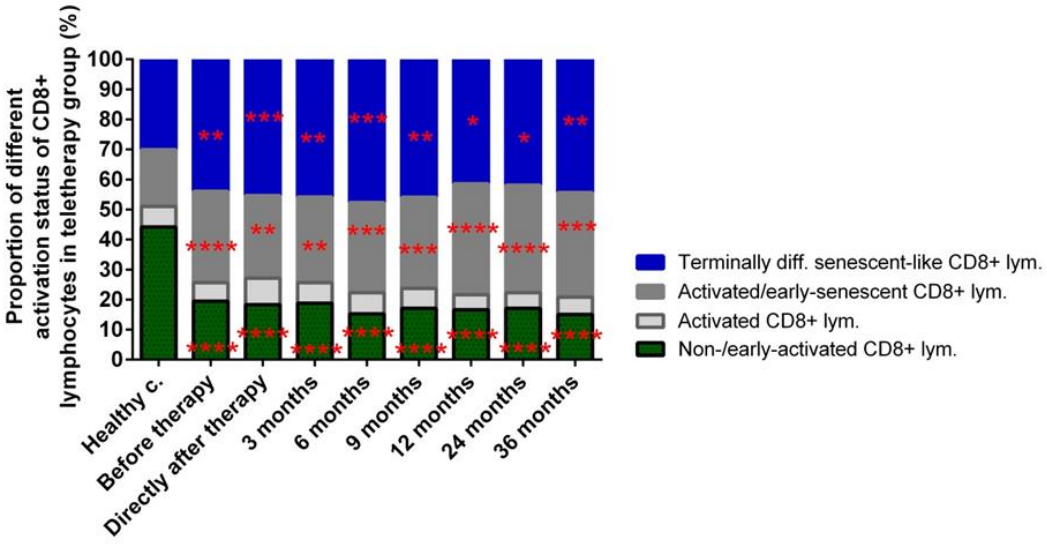

Figure 3. Changes in the fraction of the four different activation status of CD4+ (A) and CD8+ (B) T cells in control group and in prostate cancer patients received teletherapy Red asterisks represent significant changes of cancer patients compared to controls. Black asterisks represent significant changes of patients after treatment compared to pre-treatment values.

The level of CD19+IgD+ B cells within the lymphocytes did not change significantly in HDR and teletherapy groups during the 36 months follow-up compared to controls and pre-treatment values, however the different maturation and activation status of B cells show interesting changes. The level of immature B cells (named transitional B cells) significantly increased in patients 3, 6 and 9 months after therapy compared to controls and pre-treatment values, but normalized by 36 months. The level of mature B cells was lower in patients before therapy compared to healthy controls, during the follow-up of patients a continuous but not significant elevation could be seen up to 36 months. However the proportion of activated B cells and antibody presenting plasma cells significantly increased before therapy compared to control and remained at this elevated level up to 36 months.

The level of FoxP3+ regulatory T cells similarly to the other radiotherapy-treated groups were elevated throughout the follow-up up to 36 months.

Out of the analysed main PBMC categories undoubtedly NK cells were among the most strongly altered in all prostate cancer patients, nevertheless, the kinetics of changes greatly differed in the 3 patient categories. The pre-treatment fraction of total NK cells (CD16+CD56+ NK cells within the CD3- lymphocytes) was slightly higher in the HDR and teletherapy group than in LDR group. Similarly, there were variations in pre-treatment values of the individual NK subpopulations, which most probably is due to the fact that the risk category of patients was different in the different treatment categories with the LDR treatment category containing mostly low risk patients. Therapy led to significant changes in NK subpopulations depending on the type of applied radiotherapy protocol. In the LDR group dose deposition is continuous over a period of 12 months, constituting a low dose chronic irradiation of the prostate and consecutively blood doses are also very low. HDR patient receive a single high dose local irradiation. Teletherapy is a fractionated radiotherapy, where the maximum energy deposition it at the end of the fractionation protocol (approx. 5-6 weeks), nevertheless each daily fraction adds a significant contribution to the dose. Furthermore, teletherapy is also the regimen where the largest part of healthy tissue and blood volume falls in the radiation field. LDR therapy

induced very moderate changes within the NK subpopulation and the majority of these changes normalized after 36 months (with the exception of anergic NK cells, which remained below control values). Teletherapy induced a strong and persistent shift toward immature and early mature as well as anergic NK cells and a reduction in mature NK cell fraction. HDR therapy also led to a moderate increase in the fraction of immature NK cells, nevertheless it did not compromise the fraction of mature NK cells and led to a reduction in the fraction of anergic NK cells (Figure 4). These changes in the NK cells demonstrate that radiotherapy has significant and long-lasting impact on NK cell integrity and the different radiotherapy regimens depending on how energy deposition is carried out lead to very different outcomes.

A manuscript is being prepared on the effect of teletherapy on the long-lasting immune changes in prostate cancer patients. Its submission is scheduled for the first part of next year.

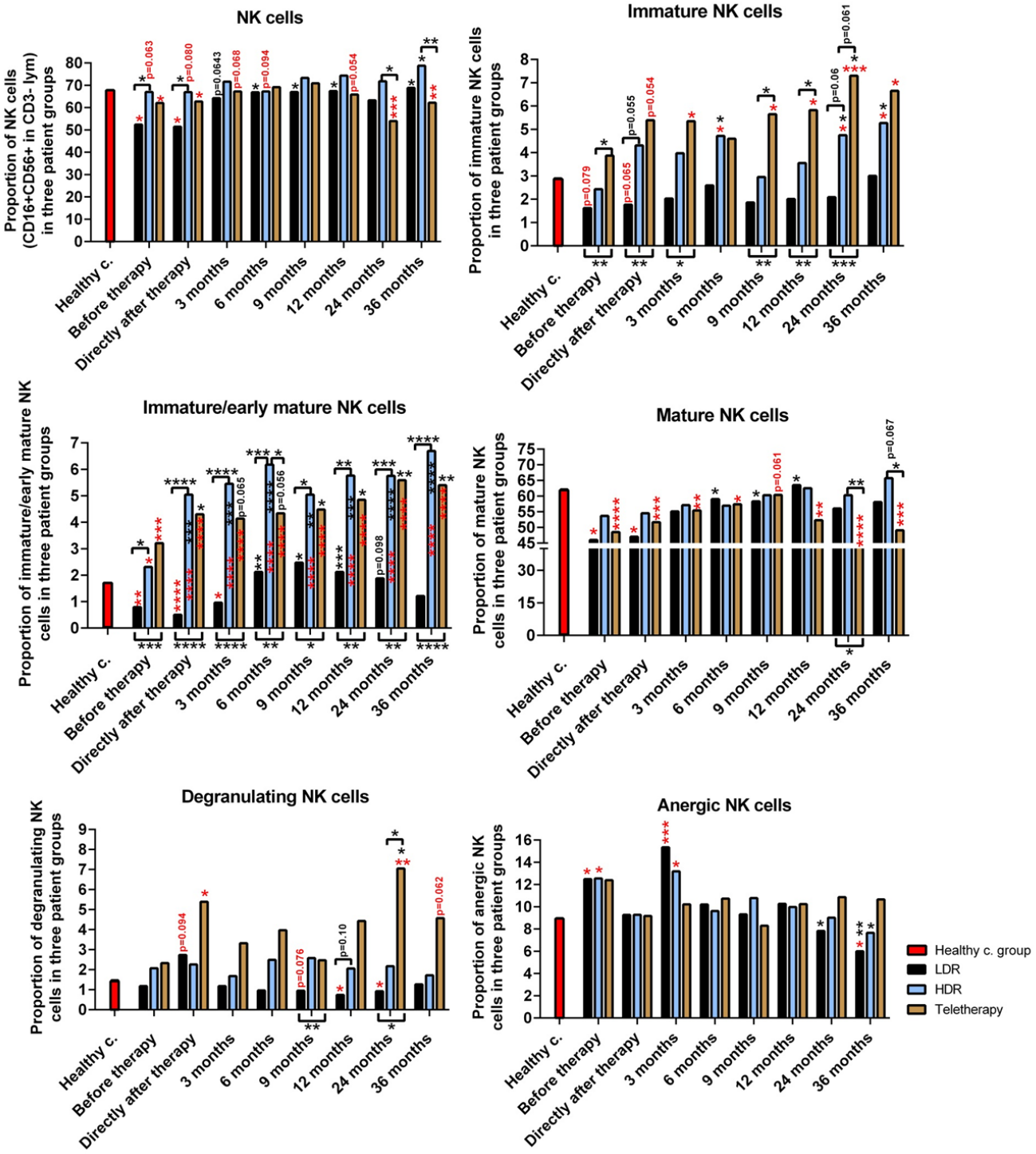

Figure 3. Comparison of the relative changes of five different maturation stages of natural killer cells in three patient groups before and after therapy up to 36 months compared to control group. Red asterisks represent significant changes of cancer patients compared to controls. Black asterisks represent significant changes of patients after treatment compared to pre-treatment values. Arrows with black asterisks represent significant changes between the patient groups.

Conclusion: Our studies demonstrate that prostate cancer patients harbour long-lasting immunological changes, part of which is due to radiotherapy, while other changes might not be directly linked to therapy. This raises the hypothesis that certain immune changes are intrinsically present in prostate cancer patients and these might constitute risk factors for the development of a malignant state. We also demonstrated that different radiotherapy protocols lead to different long-term immune alterations.

Although the project period has *de facto* finished, provided we can raise further funds to support our work we plan not stop here since these patients are being followed up in the long run to identify late therapy-related changes including therapy-related cancer (so-called second primary neoplasms) as well and to try to correlate immune changes with the late side effects. While our report contains data on patients up to three years after enrolment, patient materials are continuously being recruited and by now we have patient material from certain groups up to 5 years after therapy. Our aim would be to analyse late immune changes and to make a paper comparing long lasting immunological changes in the patients treated with different radiotherapy regimens. Such studies do not exist in the literature and would fill important research gaps in better understanding late immune sequela of cancer and its therapy.

1. Leers, M.P., et al., *Circulating PSA-containing macrophages as a possible target for the detection of prostate cancer: a three-color/five-parameter flow cytometric study on peripheral blood samples.* Am J Clin Pathol, 2008. **129**(4): p. 649-56.