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mRNA as a Platform Technology Ideally Suited for Individualized Therapeutics





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The central dogma

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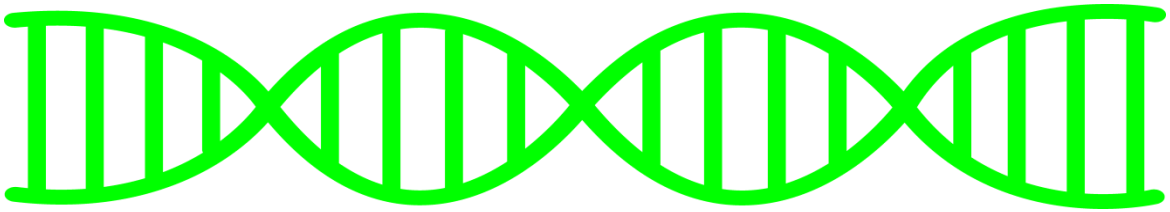
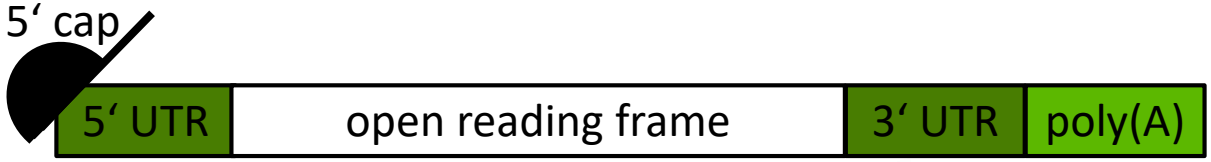
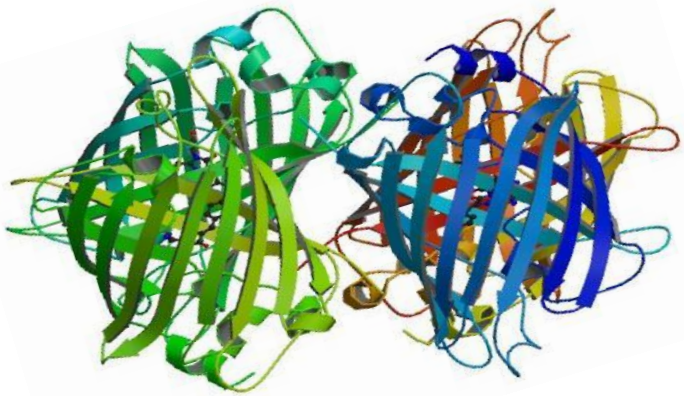
DNA



mRNA



protein



mRNA as a therapeutic

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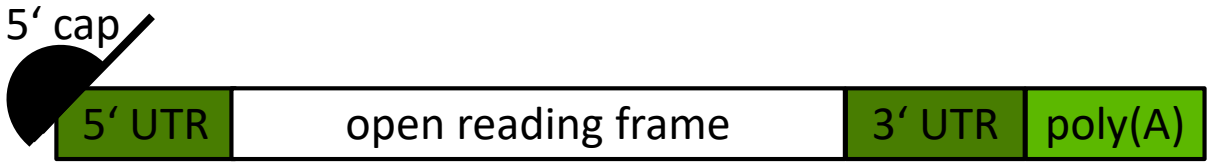
Therapeutic messenger (m)RNAs are used to introduce the genetic information for a protein, encoded by the respective mRNA, into a cell of interest



Applications for mRNA therapeutics

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Direct application of mRNA:

- Cancer immunotherapy (induction of antigen-specific T cells)
- Vaccination against viral infections
- Transcript replacement therapy

mRNA-transfected cells:

- Cancer immunotherapy (antigen-encoding RNAs into dendritic cells or RNAs coding for T cell receptors or chimeric antigen receptors into T cells)
- mRNA-induced pluripotent stem cells
- Genetically engineered cells using mRNA

Advantages of mRNA

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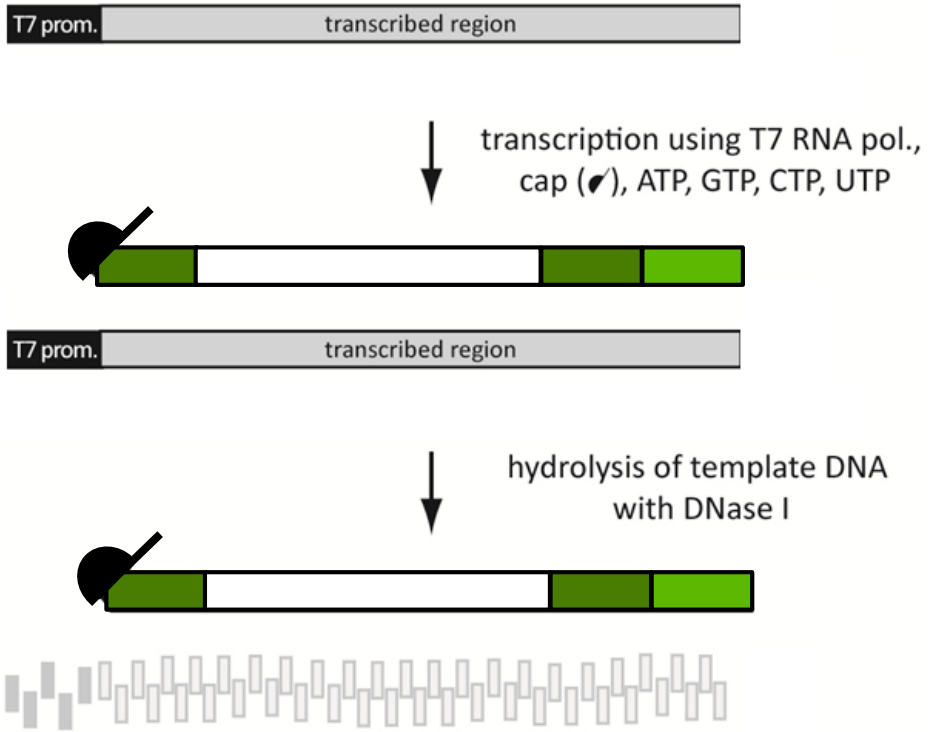
- No integration into the genome
- Needs only to reach the cytoplasm, not the nucleus
- Transient expression of the encoded protein/antigen
- Degraded into nucleotides, i.e. no toxic metabolites
- Antigen delivery independent of HLA-haplotype
- Can be used for the expression of cytoplasmic proteins
- mRNA can act as its own adjuvant (recognition by toll-like receptors, PKR and members of the RIG-I family) or can be made “immune-silent”
- Relatively simple production process independent of the sequence
- Possibility to introduce new functionalities through sequence modifications and chemically modified building blocks

In vitro mRNA transcription



linear template DNA

in vitro transcribed mRNA
(> 500 copies per template DNA)

raw reaction mixture with mRNA and impurities
(T7 RNA pol., remaining cap and NTPs,
hydrolysed DNA, …)

completely cell-free process

(but some of the starting materials come from *E. coli*)

Materials required for mRNA synthesis

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Critical starting materials

- Cap dinucleotide (building block)
- ATP, GTP, CTP, UTP (building blocks)
- Template DNA (defines mRNA sequence)

Reagents

- T7 RNA polymerase
- DNase I
- Components of the reaction buffer

mRNA purification

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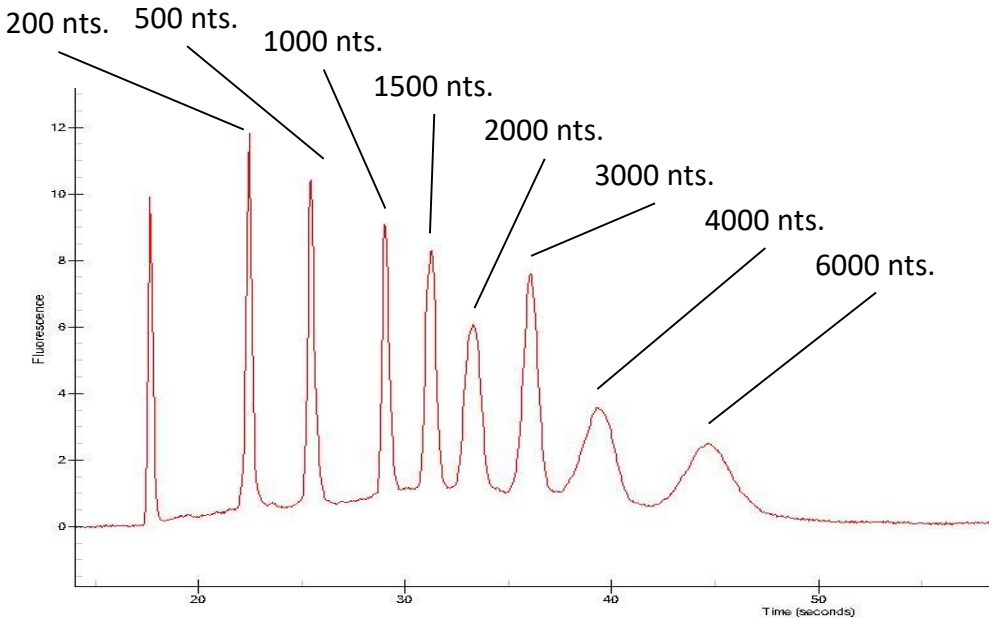
What to remove:

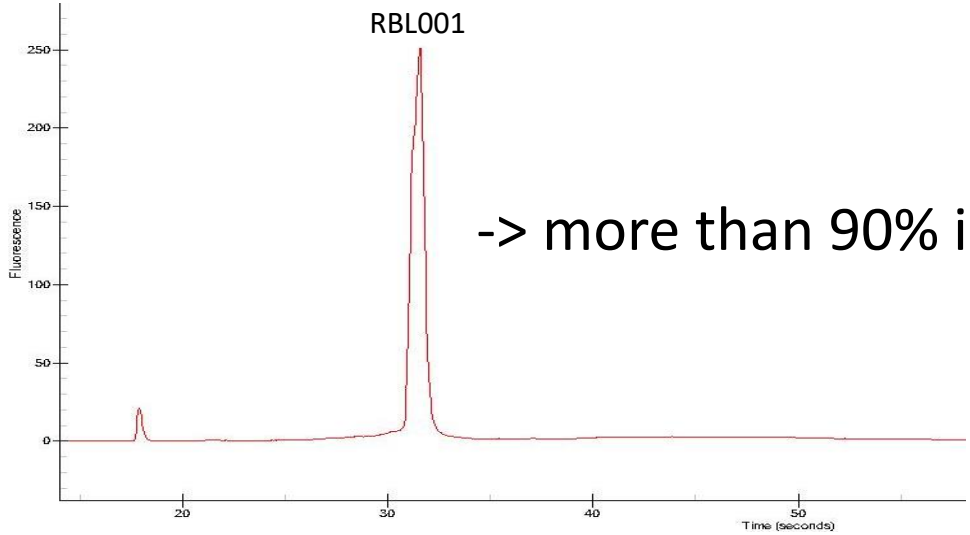
- process-related impurities (e.g. left-over NTPs, T7 RNA pol., template DNA)
- product-related impurities (e.g. break off transcripts, side products)

Challenges:

- highly charged and dynamic molecule
- RNA vs. DNA – removing the chemical cousin
- scalability of the purification process

Integrity of purified mRNA





-> more than 90% in the main peak

Quality control

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Testing each batch of mRNA for:

- concentration
- identity
- integrity
- appearance (clear, colorless)
- particles (visible/subvisible)
- pH
- osmolality
- critical residuals (e.g. template DNA)
- bioburden/sterility
- functionality/potency

Duration of the process

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- Transcription reaction: 1 day
- mRNA purification: 1 – 2 days (dependent on scale)
- Formulation, fill & finish: 1 day
- Quality control & release: 3 weeks (includes testing for sterility)

Concept of cancer immunotherapy

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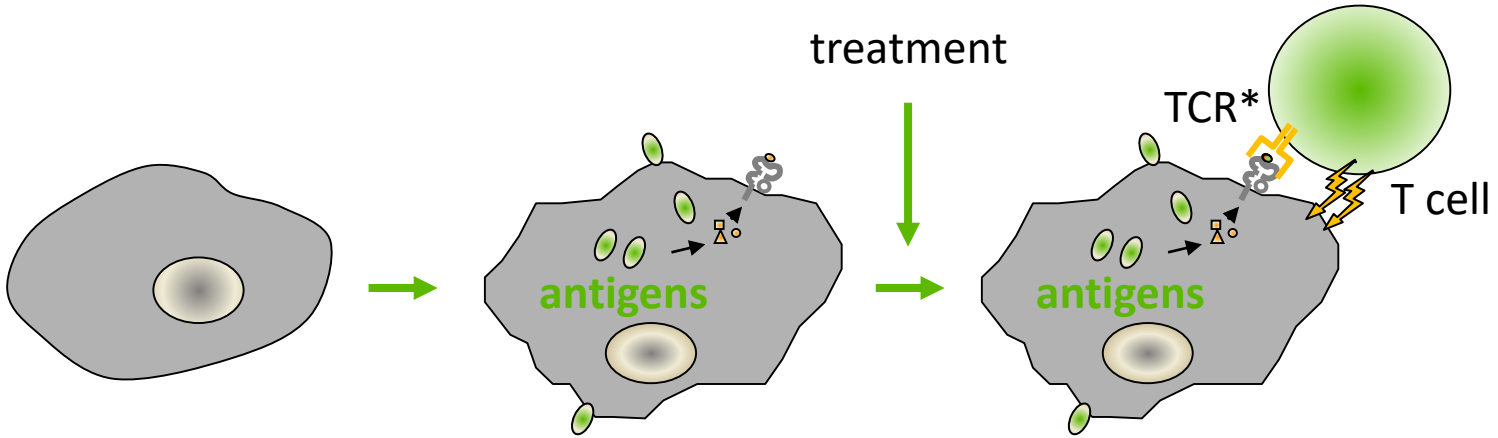
Tumor cells express tumor-associated antigens (TAAs),
which can be specifically recognized by T lymphocytes
via antigen-specific T cell receptors (TCRs)



*: T cell receptor

Induction of antigen-specific T cells

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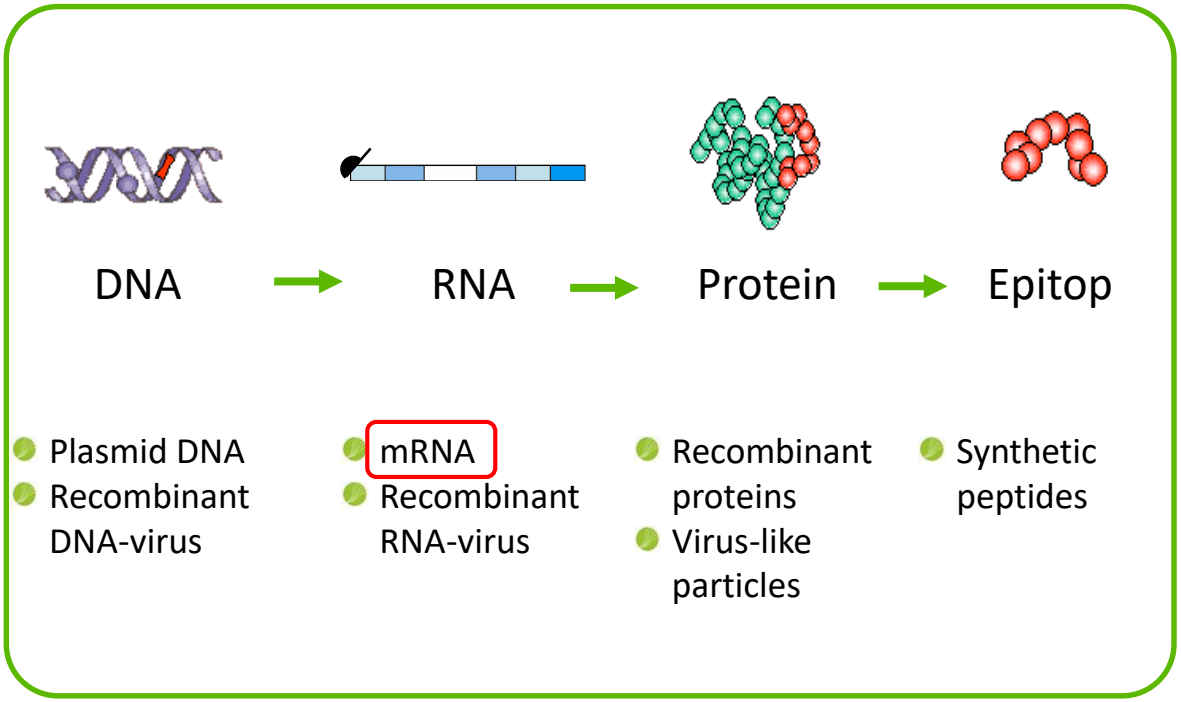
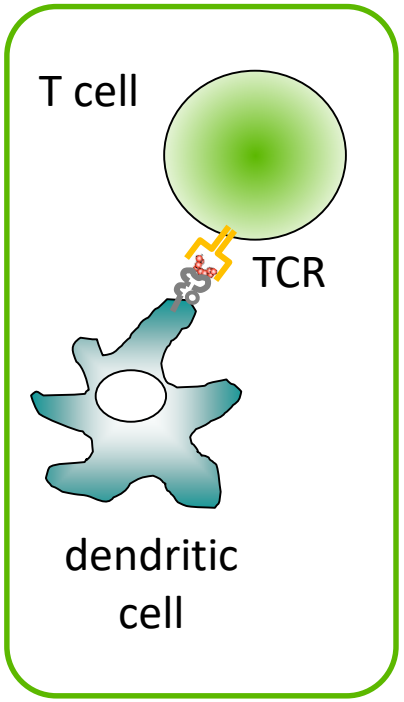
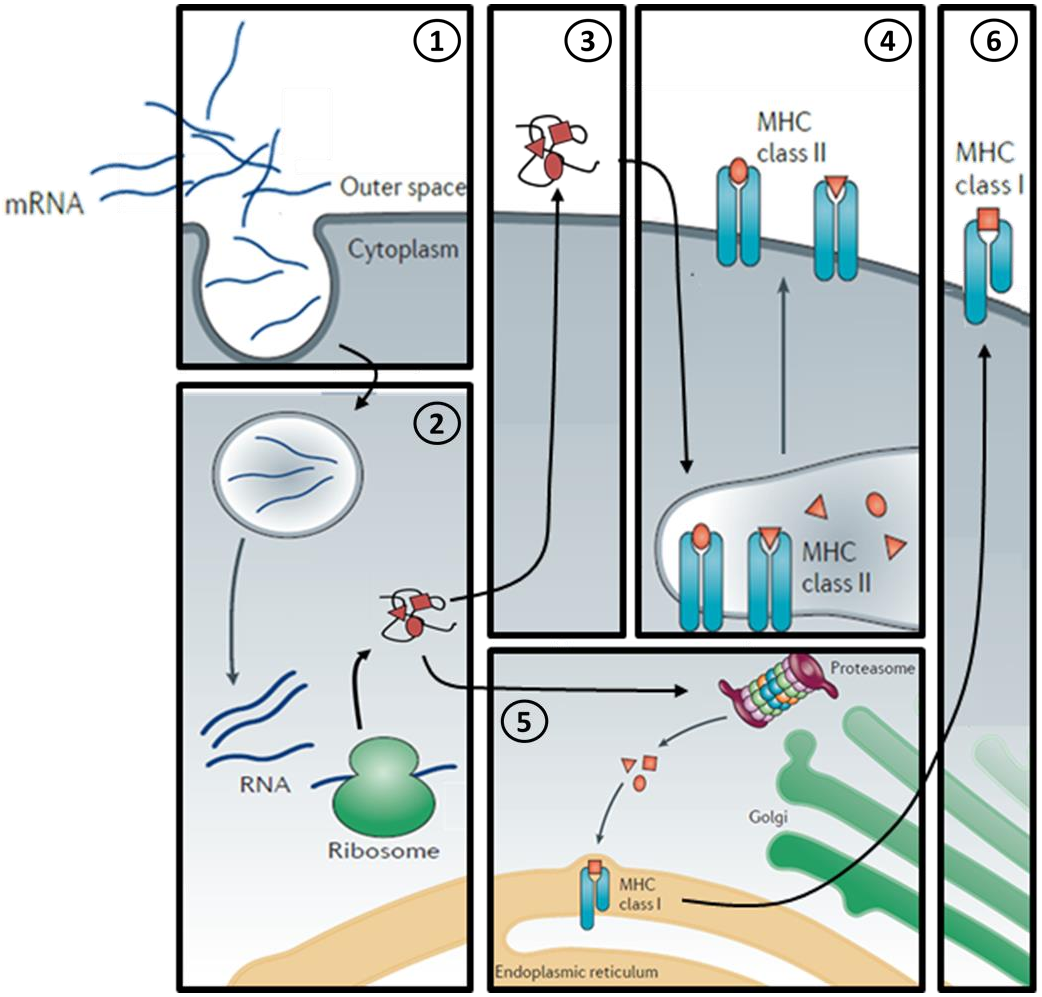
(Tumor) antigen-specific T cells can be induced by loading antigen-presenting cells (e.g. dendritic cells) with the corresponding epitopes. This can be done with different formats.





Mechanism of action



dendritic cell

- Induction and expansion of antigen-specific $CD8^+$ and $CD4^+$ T cells (via epitopes presented on MHC class I and II complexes, respectively)
- Systemic distribution
- Anti-tumoral effect

Neoepitopes as superior antigenic structures

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A vaccine targeting mutant IDH1 induces antitumour immunity

Theresa Schumacher[1,2]*, Lukas Bunse[1,2]*, Stefan Pusch[3,4], Felix Sahm[3,4], Benedikt Wiestler[1,5], Jasmin Quandt[6], Oliver Menn[1], Matthias Osswald[1,5], Iris Oezen[1,2], Martina Ott[1,2], Melanie Keil[1,2], Jörg Balß[2,4], Katharina Rauschenbach[1,2], Agnieszka K. Grabowska[7], Isabel Vogler[8], Jan Diekmann[9], Nico Trautwein[10], Stefan B. Eichmüller[6], Jürgen Okun[11], Stefan Stevanović[10], Angelika B. Riemer[7], Ugur Sahin[9], Manuel A. Friese[12], Philipp Beckhove[6], Andreas von Deimling[3,4], Wolfgang Wick[1,5] & Michael Platten[1,2]

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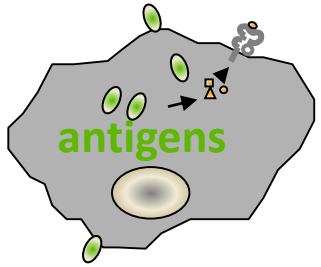
Tumor Exome Analysis Reveals Neoantigen-Specific T-Cell Reactivity in an Ipilimumab-Responsive Melanoma



tumor cell

Mutant MHC class II epitopes drive therapeutic immune responses to cancer

Sebastian Kreiter[1], Mathias Vormehr[2]*, Niels van de Roemer[2]*, Mustafa Diken[1], Martin Löwer[1], Jan Diekmann[1,3], Sebastian Boegel[1], Barbara Schrörs[1], Fulvia Vascotto[1], John C. Castle[1], Arbel D. Tadmor[1], Stephen P. Schoenberger[4], Christoph Huber[2], Özlem Türeci[1]§ & Ugur Sahin[1,2,3]§

Cancer Immunotherapy Based on Mutation-Specific CD4+ T Cells in a Patient with Epithelial Cancer

Eric Tran,[1] Simon Turcotte,[1]* Alena Gros,[1] Paul F. Robbins,[1] Yong-Chen Lu,[1] Mark E. Dudley,[1]† John R. Wunderlich,[1] Robert P. Somerville,[1] Katherine Hogan,[1] Christian S. Hinrichs,[1] Maria R. Parkhurst,[1] James C. Yang,[1] Steven A. Rosenberg[1]‡

Exploiting the Mutanome for Tumor Vaccination

John C. Castle[1], Sebastian Kreiter[1], Jan Diekmann[1], Martin Löwer[1], Niels van de Roemer[1,2], Jos de Graaf[1], Abderraouf Selmi[1], Mustafa Diken[1], Sebastian Boegel[1,2], Claudia Paret[1], Michael Koslowski[1], Andreas N. Kuhn[1,3], Cedrik M. Britten[2,3], Christoph Huber[1,3], Özlem Türeci[4], and Ugur Sahin[1,2,3]

The response of autologous T cells to a human melanoma is dominated by mutated neoantigens

Volker Lennerz†, Martina Fatho†, Chiara Gentilini‡, Roy A. Frye§, Alexander Lifke†, Dorothea Ferel†, Catherine Wölfel†, Christoph Huber†, and Thomas Wölfel†¶

Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer

Naiyer A. Rizvi,[1,2*] Matthew D. Hellmann,[1,2*] Alexandra Snyder,[1,2,3*] Pia Kvistborg,[4] Vladimir Makarov,[3] Jonathan J. Havel,[3] William Lee,[5] Jianda Yuan,[6] Phillip Wong,[6] Teresa S. Ho,[6] Martin L. Miller,[7] Natasha Rekhtman,[8] Andre L. Moreira,[8] Fawzia Ibrahim,[1] Cameron Bruggeman,[9] Billel Gasmi,[10] Roberta Zappasodi,[10] Yuka Maeda,[10] Chris Sander,[7] Edward B. Garon,[11] Taha Merghoub,[1,10] Jedd D. Wolchok,[1,2,10] Ton N. Schumacher,[4] Timothy A. Chan[2,3,5†]

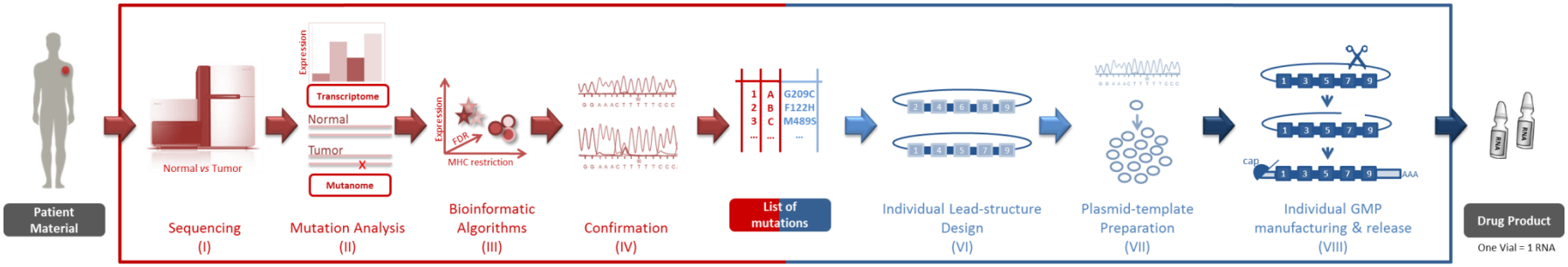
Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade

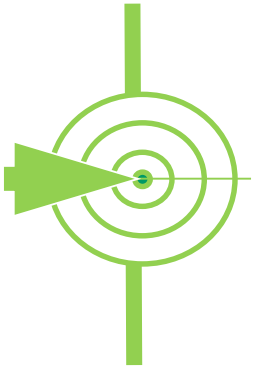
Nicholas McGranahan,[1,2,3*] Andrew J. S. Furness,[3,4*] Rachel Rosenthal,[3*] Sofie Ramskov,[5] Rikke Lyngaa,[5] Sunil Kumar Saini,[5] Mariam Jamal-Hanjani,[3] Gareth A. Wilson,[1,3] Nicolai J. Birkbak,[1,3] Crispin T. Hiley,[1,3] Thomas B. K. Watkins,[1,3] Seema Shafi,[3] Nirupa Murugaesu,[3] Richard Mitter,[1] Ayse U. Akarca,[4,6] Joseph Linares,[4,6] Teresa Marafioti,[4,6] Jake Y. Henry,[3,4] Eliezer M. Van Allen,[7,8,9] Diana Miao,[7,8] Bastian Schilling,[10,11] Dirk Schadendorf,[10,11] Levi A. Garraway,[7,8,9] Vladimir Makarov,[12] Naiyer A. Rizvi,[13] Alexandra Snyder,[14,15] Matthew D. Hellmann,[14,15] Taha Merghoub,[14,16] Jedd D. Wolchok,[14,15,16] Sachet A. Shukla,[7,8] Catherine J. Wu,[7,8,17,18] Karl S. Peggs,[3,4] Timothy A. Chan,[13] Sine R. Hadrup,[5] Sergio A. Quezada,[3,4†] Charles Swanton[1,3†]

The IVAC® process

Patient- and tumor-specific mutations get utilized…

…to selectively activate the immune system with mRNA-vaccines tailored to the individual patient.





Individualized **V**accine **A**gainst **C**ancer:

- On demand-manufacturing
- Suitable for potentially all tumor indications, also with low incidences
- No negative thymic selection of high-affinity TCRs against epitopes defined by patient- and tumor-specific mutations
- Induction of immune responses with high tumor specificity

Translation into clinical testing

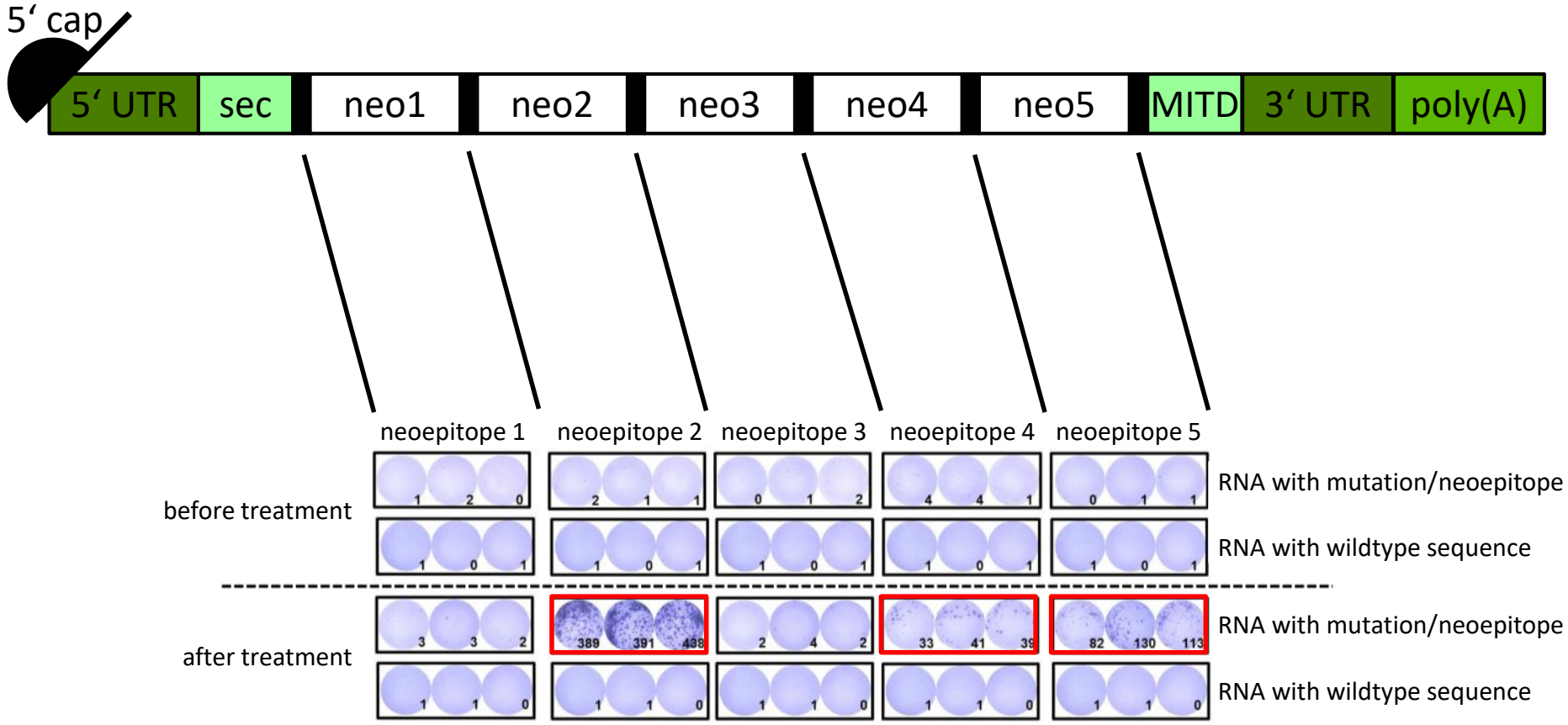
In 2013, we started the first clinical trial targeting neoepitopes defined by patient- and tumor-specific mutations:

- Indication: malignant melanoma stage IIIa - IV
- Therapy: two mRNAs encoding in total ten neoepitopes
- Dose: 0.5 or 1.0 mg of each RNA with eight applications within six weeks
- Number of patients: 13
- Primary endpoints: safety, adverse reactions and tolerability profile of multiple dosing with IVAC®
- Secondary endpoints: treatment-induced antigen-specific immune responses

Immune responses in patients

Leukaphereses before the first and after the last injection with IVAC® are acquired to provide sufficient PBMCs for a comprehensive immune monitoring program.

IFNγ-ELISpots are utilized to quantify antigen-specific immune-responses either after *in vitro*-expansion or directly *ex vivo*.



Immunogenicity of neoepitopes

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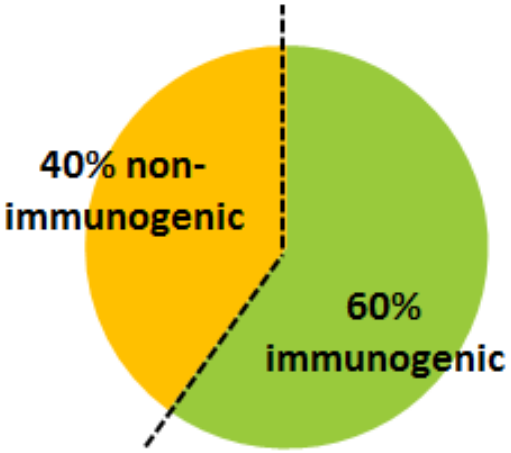
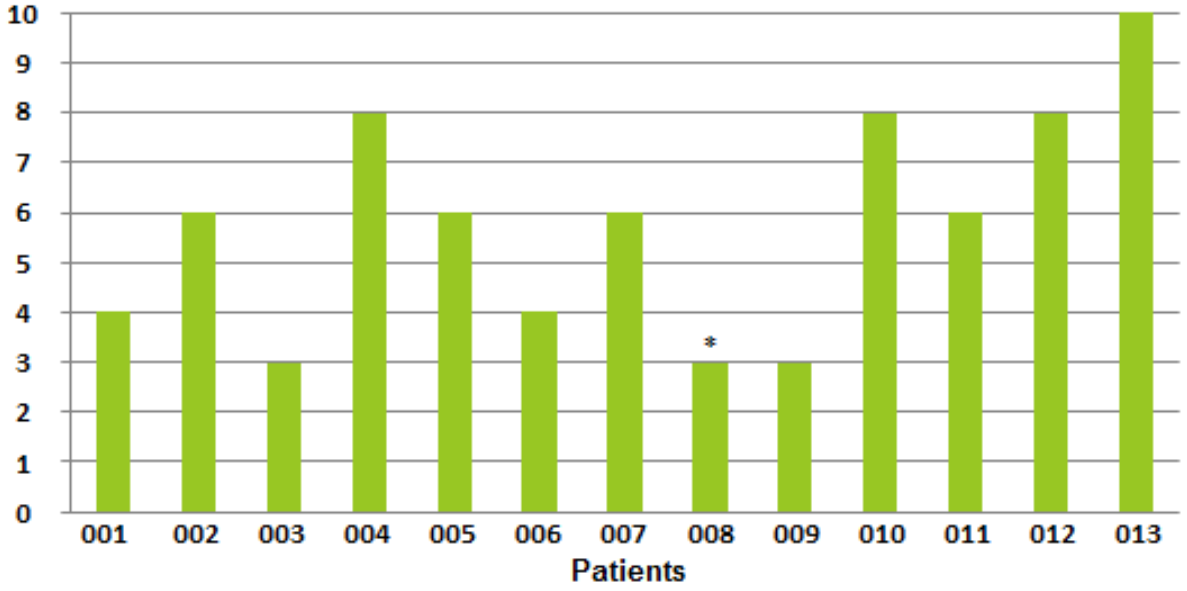
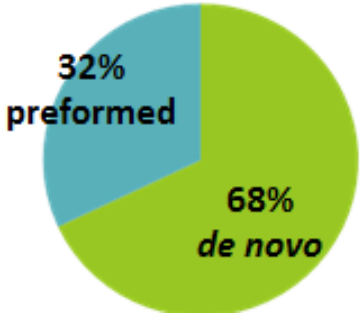
All **13 patients with a total of 125 neoepitopes** selected for the manufacture of their IVAC® RNAs have been analyzed…

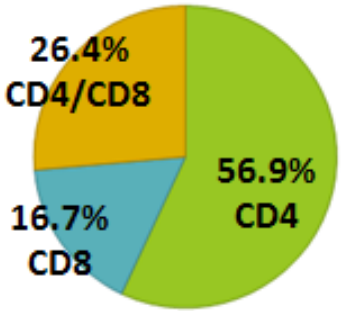




*… only 5 neoepitopes were used for vaccination

n = 75 immunogenic mutations





Summary

- mRNA is a platform technology applicable in several therapeutic fields
- Manufacturing of mRNA is fast and cost-effective; one process suitable for essentially all sequences
- Neoepitopes defined by cancer-specific mutations and encoded on mRNA (IVAC®) give rise to an anti-tumoral immune response in animal models
- A first-in-concept clinical study testing the IVAC® approach has demonstrated feasibility for a truly individualized treatment of patients
- For thirteen patients, an immune response against 60% of the neoepitopes that were targeted could be observed
- This approach is now further studied in two running clinical trials

Acknowledgements

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- The whole RNA Biochemistry & Manufacturing team
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- RNA Formulation & Drug Delivery (Heinrich Haas)
- NGS (Valesca Bukur) and Bioinformatics (Jonathon Blake)
- BioNTech Innovative Manufacturing Services (Stephan Müller)
- Project Management (Birgit Pless and Robert Jabulowsky)
- Clinical Research (Alexandra Kemmer-Brück)
- Our collaboration partners
- The patients and their families
- And many more …