View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Institute of Cancer Research Repository

Special meeting report/Special article

Starting the Fight in the Tumor: Expert Recommendations for the Development of Human Intratumoral Immunotherapy (HIT-IT)

Authors:

A. Marabelle[1], R. Andtbacka[2], K. Harrington[3], I. Melero[4], R. Leidner[5], T. de Baere[6], C. Robert[7], P.A. Ascierto[8], J-F. Baurain[9], M. Imperiale[10], S. Rahimian[11], D. Tersago[12], E. Klumper[13], M. Hendriks[14], R. Kumar[15], M. Stern[16], K. Öhrling[17], C. Massacesi[18], I. Tchakov[19], A. Tse[20], J-Y. Douillard[21], J. Tabernero[22], J. Haanen[23], J. Brody[24]

Affliliations:

[1]*Gustave Roussy, Université Paris-Saclay, Département d'Innovation Thérapeutique et d'Essais Précoces, Villejuif, France;* [2]*Surgical Oncology Department of Surgery, Huntsman Cancer Institute, University of Utah, Salt Lake City, USA;* [3]*The Royal Marsden/The Institute of Cancer Research, National Institute for Health Research Biomedical Centre, London, UK;* [4]*Clinica Universidad de Navarra and CIBERONC. Pamplona Spain;* [5]*Providence Cancer Center, Earle A. Chiles Research Institute, Portland, Oregon, USA;* [6]*Gustave Roussy, Université Paris-Saclay, Department of Image Guided Therapy, Villejuif, France;* [7]*Department of Dermatology, Institute Gustave-Roussy, Paris, France;* [8]*Istituto Nazionale Tumori IRCCS Fondazione Pascale, Naples, Italy;* [9]*King Albert II Cancer Institute, Cliniques Universitaires St Luc, Université Catholique de Louvain, Brussels Belgium;* [10] *Nektar Therapeutics, San Francisco, USA;* [11]*Idera Pharmaceuticals, Exton, Pennsylvania, USA;* [12]*Clinical Development, Bioncotech Therapeutics, Madrid, Spain;* [13]*Lytix Biopharma AS, Oslo, Norway;* [14]*Aduro Biotech, Eindhoven, The Netherlands;* [15]*MedImmune, LLC, Gaithersburg, Maryland, USA:* [16]*Roche, Zurich, Switzerland;* [17]Amgen Europe GmbH, Zug, Switzerland; [18]*Global Product Development Oncology, Pfizer, New York, USA;* [19]*Eisai Ltd, Hatfield, UK;* [20]*Oncology Early Development, Merck & Co., Inc., Kenilworth, New Jersey, USA;* [21]*Chief Medical Officer, ESMO*, Viganello-Lugano, Switzerland; [22]*Medical Oncology Department, Vall d' Hebron University Hospital, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain;* [23]*Division of Medical Oncology, Netherlands Cancer Institute, Amsterdam, The Netherlands;* [24]*Department of Medicine, Division of Hematology and Oncology, Icahn School of Medicine at Mount Sinai Hospital, New York, USA.*

Corresponding author:

Dr Aurélien Marabelle,
Gustave Roussy
114 rue Edouard Vaillant,
94805 Villejuif Cedex, France.
Tel: +33-1-42-11-55-92
Fax:
E-mail: aurelien.marabelle@gustaveroussy.fr

Manuscript statistics

Word count: 7009 excl. abstract, tables, refs, key message and conflict of interest disclosures

Figures: 1

Tables: 0

References: 34

Abstract

A European Society for Medical Oncology (ESMO)-sponsored expert meeting was held in Paris on 08 March 2018 which comprised 11 experts from academia, 11 experts from the pharmaceutical industry and two clinicians who were representatives of ESMO. The focus of the meeting was exclusively on the intratumoral injection/delivery of immunostimulatory agents with the aim of harmonizing the standard terms and methodologies used in the reporting of human intratumoral immunotherapy (HIT-IT) clinical trials to ensure quality assurance and avoid a blurring of the data reported from different studies. The goal was to provide a reference document, endorsed by the panel members, that could provide guidance to clinical investigators, pharmaceutical companies, ethics committees, independent review boards, patient advocates and the regulatory authorities, and promote an increase in the number and quality of HIT-IT clinical trials in the future. Particular emphasis was placed not only on the development of precise definitions to facilitate a better understanding between investigators, but also on the importance of systematic serial biopsies as a driver for translational research and the need for the recording and reporting of data, to facilitate a better understanding of the key processes involved.

Key words: Intratumoral, cancer, immunotherapy, consensus, recommendations

Running title: Recommendations for intratumoral immunotherapy

Introduction

The first evidence of successful cancer immunotherapy (IT) was reported at the end of the 19th century following intratumoral injections of pro-inflammatory bacterial extracts [1]. More recently, there has been a resurgence of interest in cancer IT with the success of immune checkpoint-targeted monoclonal antibodies (ICT mAbs), directed against programmed death (PD) receptor 1 (PD-1), PD ligand 1 (PD-L1) and the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), at improving clinical outcomes across a wide range of cancers. However, whilst ICT mAb therapies are limited by the small number of patients who achieve an objective response, their systemic immune-related toxicities (especially when used in combination), their cost, and the need to overcome IT resistance, are currently encouraging the exploration of other IT modalities. Intratumoral IT, as defined by direct injection of immunostimulatory agents into the tumor itself, could result in superior priming of the anti-tumor response. Furthermore, direct injection into the tumor could not only reduce systemic exposure, off-target toxicities and the amounts of drug used, but also induce stronger anti-tumor activity in the injected tumor lesion and maybe in distant non-injected tumor lesions [2-6]. Also, as described previously [2], a significant amount of preclinical rationale supports the concept that some intratumoral IT strategies may overcome resistance to ICT mAb monotherapies by priming T cells and/or allowing their intratumoral homing function. Thus, it should be stressed that these approaches although acting locally, may also help to identify systemic immune effects against cancer.

In principle, intratumoral injections can be considered for any tumor where the primary lesion or its metastases are accessible either percuteanously via direct injection or via specific procedures such as colonoscopy, cystoscopy, bronchoscopy, thoracoscopy, coelioscopy, or even surgery [7]. There is now a plethora of agents being investigated for their role in intratumoral IT, including immune receptor agonists (such as Toll-like receptor [TLR] agonists, and STimulator of INterferon Genes [STING] agonists), ICT mAbs, wild-type and genetically-modified oncolytic agents (such as viruses and peptides), cytokines and immune cells directed at a variety of potential targets [2, 8-10]. Thus, to support the clinical development of human intratumoral IT (HIT-IT) strategies, an expert meeting, comprising 11 academic experts and 11 pharmaceutical industry experts, together with two clinicians representing ESMO (the President and the Chief Medical Officer of ESMO), was convened on 08 March 2018 in Paris immediately following the ESMO Targeted Anticancer Therapies 2018 conference.

Aim

The aim of the meeting was to provide guidance and to help to structure the ongoing and future development of HIT-IT. More specifically, the objectives of this academia/industry collaborative effort were to harmonize the definitions and terms used, the methodologies, the collection of data and the reporting of results by academia and industry within their HIT-IT clinical trials. There was no discussion of particular molecules or specific trials during this meeting.

Scope

The meeting focussed exclusively on the intratumoral* injection of immunostimulatory agents. Although, some local physical and radiation strategies with known pro-inflammatory properties can be considered to act as local immunotherapies (e.g. cryotherapy, high-intensity focussed ultrasound [HIFU] and irradiation by brachytherapy/teletherapy) these strategies were considered to be outside the scope of the meeting.

*Foot note

Some strategies sometimes described as “intranodal” involve the injection of lymph nodes in the lymphoid territory draining a tumor lesion. The focus of the present work has been on intratumoral injections, with the belief that this is the best way to ensure that immunotherapies will actually reach the tumor draining lymph nodes.

Methods

Composition of the expert panel and aims

The 22 international experts were selected on the basis of their demonstrable knowledge of the field from either an academic (n=11) or pharmaceutical industry (n=11) perspective.

The aim of this ESMO-sponsored collaboration was to provide tools to help the clinical development of HIT-IT strategies by,

i) Harmonizing the definitions, terms and methods used for HIT-IT clinical trials
ii) Harmonizing the collection of data, notably the assessment of responses, during HIT-IT clinical trials, and standardizing the reporting and evaluation of HIT-IT clinical trial results.
iii) Emphasizing the importance of collecting serial biopsy and biological specimens as a driver of translational research.

Process of consensus

A draft document was prepared and circulated to the 22 experts and two ESMO representatives prior to the expert meeting. Based on the available literature and their own personal expertise and experience, the international experts were asked at the face to face meeting in Paris to endorse a series of definitions, assumptions, and proposals, and finally, to deliver a set of recommendations to support the design and management of HIT-IT trials in the future.

Results/meeting outcomes

The resulting output of this effort is the present reference document, which can be used by clinical investigators, pharmaceutical companies, ethics committees, independent review boards (IRBs), patient advocates, grant-funding organizations and the regulatory agencies to facilitate i) an increase in the quality and number of pharmaceutical industry- and academia-sponsored HIT-IT clinical trials, ii) a better assessment of HIT-IT trials leading to their approval by regulatory agencies, and finally, in the longer term, iii) the implementation of HIT-IT in the standard treatment of many cancers.

Definitions

A list of definitions was established, as outlined below, to help standardize the terminology used and facilitate a better understanding amongst those involved in this field of clinical research.

i) **HIT-IT**: Human IntraTumoral ImmunoTherapy.
ii) **Adaptive immune response:** An immune response involving antigen-specific T cells (including regulatory T cells [Tregs]) and B cells with memory features.
iii) **Innate immune response:** A stereotypic immune response from innate immune cells from both the myeloid lineage (such as granulocytes, eosinophils, neutrophils or monocytes/macrophages) and the lymphoid lineage (gamma/delta T cells, natural killer [NK] cells and NKT cells). In the context of solid tumors, these are predominantly represented by myeloid cells (there are few NK cells in the majority of solid tumors).
iv) **Anti-tumor immune priming** involves a *de novo* immune response, i.e. the generation of a novel, antigen-specific, adaptive immune response (through either B or T cells or both).
v) **Anti-tumor immune boosting** involves the enhancement of a pre-existing immune response against the tumor. This terminology can refer to i) the recall effect of a recent immune priming event or ii) the disinhibition of a pre-existing anti-tumor immune response.

vi) **In situ immunization and in situ vaccination** are alternative terms used to describe the strategy of intratumoral IT aimed at immune priming and/or boosting against the tumor. It should be noted that the term "in situ vaccination" could be confusing as it can be used for both actual intratumoral injections of a cancer vaccine as well as for intratumoral injections with immunostimulatory agents. Also, vaccination is widely understood to be a prophylactic rather than a therapeutic intervention. As such, we recommend that *in situ* immunization should be the preferred term.

vii) **Abscopal effects**: the term "abscopal" ('ab' - away from, 'scopus' - target) was proposed in 1953 to refer to the effects of ionizing radiation "at a distance from the irradiated volume but within the same organism" [11]. It is now commonly used to describe the biological or anatomical effects (such as objective radiological tumor responses) of radiotherapy outside of the field of irradiation. There was a consensus amongst the experts that the term "abscopal" was not appropriate for the description of HIT-IT in non-injected lesions. "Abscopal" is historically connoted with radiotherapy, which has specific biological effects related to its different processes in locoregional tissues and lymph nodes (e.g. multiple beam intensity-modulated radiation therapy [IMRT] can affect tissues beyond the strict tumor locus). Also, many trials are currently assessing combinations of local IT together with systemic treatments where the non-injected sites are exposed to such therapy (e.g anti-PD-L1). Therefore, we propose the novel terminology "enestic" versus "non-enestic (anenestic)" to define "injected" versus "non-injected" tumor lesions, respectively.

viii) **Enestic tumor lesions**: enestic (from "énesi" which means "injection" in Greek) was the term proposed to designate tumor lesions that have undergone intratumoral injections. Alternatively, the terminology "injected lesions" could be used.

ix) **Non-enestic (or anenestic) tumor lesions** are non-injected tumor lesions.

x) **Anenestic immune responses** are immune changes seen in non-injected lesions either regional or distant.

xi) **Anenestic tumor responses** are objective tumor responses as assessed by imaging criteria in regional and distant non-injected lesions

xii) **Tumor antigens:** includes both **T**umor**-S**pecific **A**ntigens **(TSA),** which are only expressed by tumor cells (e.g. neo-epitopes which arise from somatic mutations) and not on any other cell, and **T**umor-**A**ssociated **A**ntigens **(TAA)**, which are preferentially expressed by tumor cells but are also found on some normal cells (e.g. carcinoembryonic antigen [CEA]).

Rationale for the use of HIT- IT

HIT-IT addresses some of the limitations of the IT strategies associated with the ICT mAb therapies mentioned above, in terms of safety, bioavailability, immune priming, and achieving local effective exposure.

Safety

Intratumoral IT should minimize systemic off-target immune-related adverse events (irAEs), allowing the use of safe, synergistic combinations of immunotherapies.

Local Exposure / Bioavailability

Based on the expectation that locally-injected agents will reach high local concentrations in the injected tumor lesion, intratumoral IT offers the opportunity to increase the therapeutic index of bioactive agents injected directly into the target whilst achieving low systemic exposure. Intratumoral IT allows for an on-target therapeutic window. In addition, direct injection into the tumor may also produce a high local concentration of chemokines that helps to recruit the appropriate immune cells into the tumor micro-environment to mount an anti-tumor response.

Simplicity

In contrast to personalized vaccination strategies, intratumoral injections of immunostimulatory products provide a universally applicable tumor antigen agnostic strategy which allows the immune system to react against the most immunogenic of the full repertoire of tumor antigens, without the need for the operator to pre-determine which antigens are expressed by the tumor [12].

Types of HIT-IT

The general opinion was that every immunostimulatory medicinal agent has the potential to be injected locally as part of a HIT-IT strategy. These include the following non-exhaustive list of agents:

- **Pattern recognition receptor agonists (PRRs):** e.g TLR agonists, STING agonists, RIG-1-like receptor (RLR) agonists [13]
- **Oncolytic viruses and peptides:** e.g herpes, vaccinia, coxsackie, adeno- and reo- viruses, and lactoferrin-derived peptides [12, 14-19]
- **Immune checkpoint-targeted antibodies:** e.g tumor necrosis factor receptor superfamily (TNFRSF) agonists (e.g.OX40, CD137, GITR, CD40) [20], and Ig superfamily/B7.1 antagonists (e.g. the CTLA-4, LAG-3, TIM-3 and PD-1 immune checkpoints) [8, 21, 22]
- **Cytokines**: e.g IL-2, interferons, granulocyte-macrophage colony-stimulating factor (GM-CSF) [2, 8]
- **Immunometabolic modulators**: e.g indoleamine dioxygenase inhibitors, adenosine receptor (A2AR) inhibitors, anti-CD73 [2, 8]
- **Encoding nucleic acid sequences:** e.g cytokine encoding mRNA [2, 8]
- **Immune system cells:** e.g dendritic cells, chimeric antigen receptor (CAR)-T cells, [2, 8]
- **Nano or microparticles** [2, 8]
- **Bispecific T-cell engaging antibodies** [2, 8].

Pharmacokinetic evaluation of HIT- IT

Pharmacokinetic (PK) data remain essential for HIT-IT strategies, particularly for novel agents in order to establish not only the systemic exposure to, and the half-life of, the agent, but also to build a case for its safety.

It can be envisaged that the PK of the agents involved in HIT-IT procedures may be impacted by the tumor vasculature, the ratio between tumor size and the volume of injection, tumor interstitial pressure, the volume and concentration of the agent, the level of expression of the agent's target in the tumor, the reversibility of binding of the agent to its target (off rate) or to other intratumoral molecules (e.g. potential slow release if partially liposoluble), and any local metabolism of the IT agent. The PK may also be impacted by features of the host such as anti-drug antibodies and anti-agent cellular responses (e.g. phagocytosis of oncolytic viruses).

Thus, any PK analysis of HIT-IT trials should include time points that are very close to the time of the IT injection as well as those that allow assessment of long-term exposure over the subsequent hours and days. Also, the number of patients tested needs to be significant in order to properly assess the potential inter-individual variability. To monitor the systemic PK of any locally injected agent, it will also be necessary to demonstrate that the effects on non-injected/anenestic tumor lesions are not due to any significant systemic exposure to the agent. In addition, tissue PK analyses should be performed whenever possible (e.g. neo-adjuvant/window of opportunity settings) in order to establish data on the local concentration, the volume of distribution and time on target, of the injected agent.

It should be noted that traditional pharmacokinetic studies investigating absorption, distribution, metabolism, elimination, and drug-drug interactions are not relevant in the evaluation of intratumoral

oncolytic virus therapies. Non-clinical studies have instead focused on the biodistribution and clearance, shedding, and replication in normal and tumor-bearing mice.

Pharmacodynamic evaluation of HIT-IT

The development of pharmacodynamic (PD) endpoints will be critical for the development of HIT-IT, especially the identification of biomarkers that will allow us to correlate local activity with systemic efficacy. Depending on the drugs used, these could include (non-exhaustive list):

- Treg depletion/modulation
- Recruitment, activation and/or expansion of anti-tumor T cells (T-cell priming)
- Activation of anti-tumor B cells (B-cell priming) and generation of tumor-targeting antibodies
- Recruitment and presentation of antigen presenting cells (APCs): Dendritic cells (DCs), macrophages, B cells, and also human leukocyte antigen (HLA)-I and/or HLA-II expression by tumor cells
- Macrophage depletion versus activation or M1/M2 differentiation
- Enhancement of the antigen-presenting properties of cancer cells (HLA, co-stimulatory/co-inhibitory molecules)
- Increased cross-priming of tumor antigens (TSA and TAA) in the tumor microenvironment or tumor draining lymph nodes.
- Emergence of new T-cell receptor (TCR) clones as a measure, albeit non-specific, of an expanding repertoire post-treatment
- Generation of specific circulating immune cells (e.g. proliferation of CD8 + PD1 high T cells)
- Generation of memory T- and/or B-cell responses
- Development of tertiary lymphoid structures in tumors
- Expression of adhesion molecules by endothelial cells
- Tumoricidal effects
- Local/distant impact on the patient microbiome.

Going forward, PD studies should be built on the collection of systematic pre-treatment and on- or post-treatment biopsies of both enestic and anenestic tumor lesions. Early biopsy time points (i.e. prior to the first disease assessment) in both responders and non-responders should be considered including those who had documented disease progression (who often do not undergo tumor biopsies when disease progression has been clinically or radiologically documented). Currently, the ideal time points and targets for PD sampling are unknown but will most probably be dependent on the mechanisms of action of the agents or drugs used. As with PK, we recommend that HIT-IT study design, as best practice, should also include the analysis of systemic PD target effects at the same sampling time points as the tumor biopsies. The timepoints for PD sampling should rely on pre-clinical data or be based on their anticipated immune modulatory effects. Repeated PD sampling might be necessary during the early stages of development to assess the kinetics and amplitude of the immune responses, and also any bell-shaped curve effects due to physiological negative feedback loops.

Intratumoral injections also offer the opportunity to perform longitudinal studies with a tumor biopsy or fine needle aspirate (FNA) taken during every intratumoral procedure, and repeated at progression or during response. In addition, neoadjuvant, window-of-opportunity designs offer the possibility of generating data on the local impact (i.e. surgical specimen and sentinel lymph nodes) of HIT-IT. The safety and very low systemic exposure of some HIT-IT strategies may allow us to classify trials of intratumoral injections as phase 0 clinical trials.

The prerequisites for the translation of the local immune reaction into efficient systemic anti-tumor immunity are still not fully understood. Thus, tumor biopsies are critical to our better understanding of

the *in vivo* PD of immunotherapies. It is recommended that quality checks become part of the standard operating procedures for tumor biopsies (e.g confirmation of the actual presence of tumor cells in a target lesion at baseline). Clinical trial case report forms (CRFs) should be used to collect all the details relating to the biopsied sites. Indeed, the interpretation of translational studies might be impacted by the microenvironment of the tissue where the cancer is located. Therefore, it is recommended that the locations of all biopsies should be captured on the patients' CRFs (e.g. lymph node versus muscle versus liver).

It was also recommended that pre-treatment tumor biopsies should be performed during the trial screening period, and not at the time of the first intratumoral injection, in order to better distinguish procedure-related adverse events (AEs) from treatment-related AEs. However, for longitudinal studies, tumor biopsies could be performed alongside intratumoral injections.

PD studies and the resolution of some unsolved questions

In particular, there is an urgent need to generate data regarding the generation of a systemic immune response. The unsolved questions include:

- Are pre-existing immune infiltrates (and their phenotype) predictive of the efficacy of HIT-IT?
- Are there specific combinations of HIT-IT which could prime/enhance anti-tumor immunity?
- Is the quality (i.e. phenotype) of the tumor immune infiltrate more important than their topography (i.e. location in the tumor) or are they both important?
- Are some tumor histiotypes or tumor sites/subsites more sensitive to HIT-IT?
- Are there primary mechanisms of resistance which prevent patients from responding to HIT-IT?
- Does HIT-IT deliver drugs better to, or have more impact on, tumor draining lymph nodes than systemic treatment?
- Are some tumor histotypes and tumor sites better than others to prime an anti-tumor immune response against non-injected sites?
- Are some tumor histotypes and tumor sites better than others to generate an objective radiological tumor response in non-injected sites?
- Can HIT-IT change a cold (non-inflamed) tumor into a hot (inflamed) tumor?
- Are typical biomarkers associated with systemic IT efficacy also correlated with the efficacy of HIT-IT (e.g. PD-L1 expression or tumor mutational burden)?
- Does HIT-IT require combination with systemic treatment (e.g. ICT mAbs)?

Clinical Goals of HIT-IT

The clinical aims of HIT-IT depend on the type of clinical trials (Phase 1, 2 or 3), and include the achievement of:

- Local efficacy, i.e. an objective response at the site of injection (target tumor site), with special interest in the ability to generate enestic (local) complete responses, is of relevance especially for tumors that are invading into adjacent vital structures such as is seen in head and neck squamous cell carcinomas
- Systemic efficacy as evidenced by anenestic responses, including a clear definition and demonstration of the occurrence of such activity (to be discussed further below)
- "Drug escalation" at the site of injection rather than dose escalation (i.e. an increase in the number of locally-delivered drugs with the aim of seeking synergistic combinations rather than searching for an optimal dose)
- Reversal of resistance (intrinsic primary resistance and adaptive or acquired secondary resistance)

- The treatment of sanctuary sites (where it is difficult to get a sufficient concentration of chemotherapy or infiltration of immune cells; e.g. bone and ovaries, respectively), or sites of oligoprogressive disease, as part of a more generalized response
- The provision of symptom relief
- Down-sizing and reducing the recurrence of tumors in the neo-adjuvant setting.

The unresolved clinical questions of HIT-IT

In achieving these clinical aims, there are many important questions that remain to be further explored and resolved. These are as follows:

- Does injecting a metastasis versus a primary tumor influence the efficacy of HIT-IT?
- What are the limiting factors in the achievement of a distant anenestic response? Is this dependent on soluble factors, the homing of T cells, local T regs or the presence of macrophages, or other APCs?
- Is the concomitant injection of numerous tumor lesions better than the sequential injection of an increasing number of tumor lesions over time, in order to target as many sources of antigenic diversity as possible and generate true polyclonality of the anti-tumor response?
- How do the treating physicians prioritize which tumor lesions to inject if only a limited volume/dose can be administered at each treatment visit? Should prioritization be made according to tumor size, beginning with the largest lesion, or alternatively involve the injection of any new injectable lesion that has appeared since the previous injection?
- Does the sequential injection of multiple tumor lesions generate a priming or boosting effect?
- Does neo-adjuvant HIT-IT protect against post-surgical relapses?
- Does HIT-IT generate a better memory anti-tumor immune response than systemically administered immune therapies?
- How long do we need to continue to inject locally? How do we decide when to stop?
- When an injected tumor lesion achieves a complete response (CR), should we inject other non-injected lesions?
- Should concomitant lymphadenectomy be performed or is lymphadenectomy harmful for the long-term effect of HIT-IT (e.g. neo-adjuvant settings)?
- Can more than one HIT-IT agent be co-injected in the same lesion achieving synergistic efficacy?

Long term (>6 month) radiologically stable disease might sometimes be explained by scars secondary to histological necrosis. Thus, we recommend biopsying tumor lesions which become durably stable upon HIT-IT in order to document the ongoing biological changes (including potential necrosis) and to inform our understanding of the overall biological processes involved in injected and non-injected lesions. We also recommend biopsying tumor lesions showing dissociated (mixed) responses, or new tumor lesions, in the context of overall disease control, for the same reason.

Intratumoral injections and injectability

Several parameters can impact the development of HIT-IT as a therapeutic strategy such as the choice and size of the target tumor lesion, the accessibility of that lesion, the conspicuity of that lesion and the availability of the appropriate imaging techniques to properly inject/assess those lesions.

Tumor site and location

The experts presented their opinions on tumor site and location as follows. All tumor sites are potentially injectable, but injection at some tumor sites might require the support of additional clinical/surgical/radiological specialities and specific technologies in order to achieve accurate injection of the target lesion. These include the use of ultrasound, computed tomography (CT)- and

magnetic resonance imaging (MRI)-guided injections, colonoscopy, cystoscopy, bronchoscopy, thoracoscopy, coelioscopy, and surgery.

Some visible or palpable tumor lesions, depending on their location, can be injected without image guidance. Indeed, within current clinical practice using approved intratumoral compounds, local treatment of superficial lesions does not require any specific image guidance:

- Skin and sub-cutaneous lesions
- Superficial lymph nodes (e.g. inguinal, sub/supraclavicular, cervical)
- Mucosal lesions (e.g. oral, anal, cervical),

There are tumor lesions that, depending on their location, will require imaging guidance in order to be injected for example:

- Lung lesions
- Liver lesions
- Deep (e.g. retroperitoneal, pelvic, thoracic) lymph nodes.

Tumor lesions, at specific locations, could require endoscopic guidance in order to be injected. Such lesions include:

- Endobronchial lesions (e.g. non-small cell lung cancer)
- Endoluminal lesions (e.g. colorectal cancer)
- Endosinusal lesions (e.g. head and neck squamous cell carcinoma).

Also, it should be noted that some tumor lesions can only be accessed via surgical procedures, for example most peritoneal lesions and central nervous system tumors. As a consequence, these lesions cannot undergo multiple local injections unless a means of inserting a delivery port at surgery can be safely defined.

Most superficial tumor lesions (cutaneous, subcutaneous and superficial lymph nodes) can be injected under ultrasound guidance. Accessible liver metastases can also be injected using ultrasound guidance. Within clinical trials, ultrasound guidance is recommended not only to guide the positioning of the needle in the tumor lesion, but to allow for tumor measurements (tumor dimensions) at every injection in order to better monitor the kinetics of response/progression and local tissue changes (e.g. necrosis or hematoma). However, this recommendation does not apply to post-approval routine practice when lesions can be visually injected. Doppler ultrasound is recommended for injections with agents where there is a potential risk of systemic exposure, to ensure that the injection is not performed within a vessel.

Ultrasound-guided injections of deep tumor lesions should be offered to patients whenever the procedure seems feasible, in order to avoid the burden of CT-guided procedures (i.e. repeated X-ray exposure and longer procedures). However, CT is mandatory for the injection of lung tumor lesions.

Tumor size limits

Within clinical trials, injected tumor sites should measure ≥1 cm in diameter (≥1.5 cm for lymph nodes) to ensure injectability. For skin and subcutaneous lesions where confidence in achieving intralesional delivery is higher, smaller diameters might be eligible. Within clinical practice for approved compounds, there might be no tumor size limits, most notably for the local treatment of superficial tumor lesions.

Image-guidance

Image-guidance essentially involves three different steps, guidance of the needle, assessment of the needle's location prior to delivery, and the post-injection assessment of drug delivery.

Guidance of the needle is a relatively standard procedure and can be left to the operator. The assessment of the position of the needle can be captured and monitored (using screen shots) during standard radiological procedures (ultrasound- or CT-guidance). The needle position should be specified (e.g. central, peripheral or peritumoral) and may involve multiple positions during the same procedure (e.g. clockwise in the tumor at positions 12, 3, 6, and 9 o'clock, or similar to local anesthesia using one injection to apply multiple depots in a prespecified confined area). Finally, a rigorous assessment of drug delivery requires that the drug can be seen with standard imaging techniques or co-injected with a radio-opaque product and then monitored. Such studies have shown that the distribution of drugs in a tumor lesion can be highly non-homogeneous [23-27]. However, some HIT-IT agents might be affected by radio-opaque products which could make such co-injection detrimental.

CT scan image capture allows for external review, which ultrasound does not. Both techniques allow for the acquisition of 3D volume data. Factors associated with the success of image-guided needle biopsies of tumors have been published elsewhere [28].

Expertise in interventional radiology, contrast-enhanced ultrasound and CT-scan availability for the procedures described above, are all practical considerations for selecting centers for HIT-IT clinical trials.

Needle size and length

Needles are available in a range of lengths and gauges (diameters) for the delivery of drugs, vaccines, and other substances. For intratumoral injections involving a deep lesion a 22 gauge (0.72 mm) needle should be used (as a thinner needle is difficult to manipulate in deeper lesions), but for skin lesions, needles as small as 30 gauge (0.31 mm) may be used [28]. For tumor biopsies, an 18 gauge needle (1.27 mm) is recommended, with the collection of up to 6 cores possible per procedure.

Syringes

Only Luer lock syringes should be used in order to avoid leakage and accidental dislodgement of the needle during intratumoral injections. No specific needle or device has currently demonstrated its superiority in terms of therapeutic efficacy or pain relief.

Volumes of injections

For skin and mucosal lesions, depending on the volume of injection (e.g. >200uL), increased interstitial pressure and tumor size can increase the risk of extravasation (back spilling), resulting in less control over the actual local delivery of the agent. In general, for deep lesions, a minimum volume of 500uL is recommended to ensure better control of the delivery. Every intratumoral procedure and pharmacy preparation should take into account the dead space of the needle to ensure that the actual prescribed dose is delivered to the target lesion(s).

Number of lesions injected

Multiple lesions can be injected during the same procedure. This could involve the same needle being used for multiple site injections within the same patient, which would prevent syringe manipulation, drug spillage and drug exposure to the patient and the operator during the injection procedure. Alternatively, one syringe/needle could be prepared for each lesion to be injected.

Operators

Any trained nurse, doctor, radiologist, interventional radiologist or surgeon can perform intratumoral injections.

Procedures

Local (at site of injection) plus or minus systemic analgesic treatments should be anticipated and initiated at least 30 minutes prior to any painful HIT-IT procedure. Skin analgesia at the site of injection can be administered using topical xylocain (4%) or other local anaesthetic agents. The options for systemic analgesia should include the full range of analgesia from paracetamol/acetaminophen to opioids, depending on the precise details of the procedure and the patient's underlying symptoms. Preference should be given to the use of the thinnest needle available to minimise morbidity and limit leaks from multiple punctures. Ideally if multiple parts of the same lesion need to be injected, a single puncture entry point to the tumor lesion is preferred. For large lesions, intratumoral injections should be preferentially targeted towards viable tissue near the periphery of the tumor, and should not be injected into the necrotic core. Also, for tumor lesions that are too dense or hard to penetrate with a needle, injections should be delivered around the periphery of the lesion. As mentioned above, a fan-shape injection technique should allow better diffusion of/exposure to the injected product in the tumor lesion. However, research is needed to optimize the tools for delivering local IT. The added value of a needle with multiple side-holes or a multi-pronged needle remains to be demonstrated.

As the impact of the needle trauma on the tumor immune microenvironment is unknown, sham procedure studies (e.g. an intratumoral injection of saline) are needed to evaluate any potential impact. Similarly, the impact of local/systemic anesthetics on immune cells *in vivo*, or on the stability of the injected agent, also remain largely unknown.

Patient exclusion criteria

Use of anti-coagulant agents or history of significant bleeding diathesis

Patients on agents such as non-steroidal anti-inflammatory drugs (NSAIDs), aspirin, or clopidogrel are eligible to receive HIT-IT and these agents do not have to be withheld. For procedures with moderate or significant risk of bleeding (deep lesions and/or organs), long-acting agents such as aspirin or clopidogrel should be discussed on a case by case basis (tumor board, principal investigator, medical monitor, or standing policies of the IR group). Patients with therapeutic doses of anticoagulants, should be excluded from most deep lesion biopsies and injections. However, for deep injections in patients receiving a preventive dose of low molecular weight (LMW) heparin it is recommended that their LMW heparin treatment is stopped 24 hours prior to the intratumoral injection and resumed again 24 hours after the injection. A minimum platelet count of 50,000/mm^3 is recommended for patients being injected in deep tumor lesions. No specific coagulation and platelet restrictions should be applied to patients with skin, sub-cutaneous and superficial lesions where mechanical hemostasis can be easily implemented.

History of severe allergy to the injected agents

Patients with a known severe allergy (Common Terminology Criteria for Adverse Events [CTCAE] grade 3-4) to the injected agents should be excluded from such a therapeutic strategy. This exclusion should only concern patients who have experienced actual anaphylactic reactions (i.e. IgE-mediated events). Patients with a history of anaphylactoid reactions (i.e. mastocyte degranulation) are not excluded but it is suggested that such patients should be treated with caution and, perhaps, a more prolonged period of observation in the hospital setting after the first 1-3 injections.

Risk of vascular catastrophe

Lesions in the vicinity of large vessels with a risk of vessel blow out (e.g. the common, internal or external carotid arteries or their branches), or other situations with a risk of vascular catastrophe such as tumor-encased large vessels should be excluded from HIT-IT. Notably, special caution should be taken in patients with neck lesions that have been re-irradiated, especially if the second course of

radiation was given at radical doses with curative intent, and in whom the disease is ulcerated and/or connects to a skin or mucosal surface. Also, patients with tumor lesions with macroscopic intravascular tumor invasion (e.g. liver lesions with tumor infiltration into the main portal vein, hepatic vein or vena cava) should not receive intratumoral therapy.

Dose/regimen

Rationale for the choice

Typical *de novo* immune reactions, against pathogens/vaccines for example, include a priming phase with intense immediate, stereotypic, innate immune activation for several days in a row followed by the generation of a slower, antigen-specific, adaptive, T cell- and B cell-mediated, immune response. This step can be associated with fever, which is a physiological sign of the systemic immune reaction whose intrinsic features and properties contribute to the efficacy of the immune reaction. Therefore, fever *per se* should not be treated or prevented unless it is not well tolerated by the patient (e.g. elderly patients). If necessary, patients can receive antipyretic medications, such as paracetamol/acetaminophen, and nonsteroidal anti-inflammatory drugs such as naproxen, diclofenac, or ibuprofen. Subsequent re-exposures to the same antigens have fewer systemic effects and mostly rely on the adaptive immune system. Every re-exposure to the antigen results in an enhancement of the adaptive immune response against the antigen (i.e. as a boosting effect).

The ideal dose or regimen for HIT-IT has not yet been determined for any immunostimulatory agent and is expected to vary across agents depending on their mechanisms of action and their local PK/PD properties.

The conventional rules for drug development such as identifying dose-limiting toxicities (DLTs) and maximum tolerated dose (MTD) might not be appropriate for HIT-IT trials. Instead, beyond safety assessment, phase 1 HIT-IT clinical trials are needed to determine the optimal active dose/regimen with either specific biomarkers or early radiological assessments. Also, the every 2-week and every 3-week regimen/cycles inherited from systemic chemotherapy trials as a strategy for managing the cyclical toxicity of cytotoxic agents, are not relevant for immunostimulatory agents. Thus, HIT-IT trials with innovative regimen and schedule designs are to be encouraged.

Dose per injection

Doses per injection timepoints can be determined on a per lesion basis (fixed dose/volume or adapted to the size of lesions), on a tumor load basis or on a per patient basis (fixed dose per patient versus per body weight dose). Which dosing paradigm to pursue might depend on the predominant toxicity of the IT agent. For example, a dose per lesion (i.e. fixed concentration) approach might be more appropriate if injection-site reactions are dose-limiting, whilst a dose per patient (i.e. fixed total dose) approach would be more suitable if systemic toxicity prevails. The ratio between the volume of injection and the injected lesion/tumor size may be critical for the activity of the HIT-IT. Therefore, the details of the size of the injected lesions and the actual injected volume of the IT agent should be collected during clinical trials on the CRFs.

Priming

Depending on the local half-life and/or the duration of the local bioactivity of the immunostimulatory agents, several intratumoral injections might be necessary for the local priming of the anti-tumor immune response. Therefore, a certain dose-intensity might be useful during this initial induction phase. However, some tumor sites might be less amenable to multiple injections. The minimal number of injections, per tumor lesion, needed to obtain priming remains undetermined. Also, it is not known if the initial injection of multiple lesions generates a better priming response than the injection of a single lesion. Evaluation of these parameters in early-phase clinical trials is encouraged, since

this knowledge may allow the more rational application of a specific HIT-IT agent – and may contribute to a general understanding in the field.

Boosting

Based on the experience of adaptive immune responses against pathogens and anti-infectious vaccines, repeated intratumoral injections at least 3 weeks apart might enhance the adaptive immunity against the tumor. The minimal number of injections needed to achieve boosting also remains undetermined.

Prime-boosting

A prime-boosting effect could be achieved by injecting multiple tumor lesions over time, or by modifying the type of agent used for HIT-IT (e.g. two different oncolytic viruses).

Dose escalation versus drug escalation

During conventional drug development, a classical dose-escalation trial design aims to identify the MTD of an investigational agent based on the incidence of DLTs, in order to develop the agent further at the recommended phase 2 dose (RP2D). During intratumoral IT, high concentrations of immunostimulatory agents can easily be reached in the absence of systemic toxicities, and, therefore, without DLTs. Therefore, the aim is to identify an optimal bioactive dose which, together with the low level of systemic toxicity, allows for a drug-escalation rather than a dose-escalation trial design. In other words, the focus is no longer on defining the safety of a systemic monotherapy dose escalation but rather to study the safety of local combinations of immunostimulatory agents, each being tested at a flat optimal bioactive dose.

Lesion escalation versus dose-intensity escalation

In order to better address the heterogeneity of cancer, one HIT-IT strategy could consist of injecting as many tumor lesions as possible to prime the anti-tumor immunity against as many neo-antigens as possible. Ideally, once the RP2D of a HIT-IT agent has been defined, subsequent development would include small-scale, randomized, comparison of single- versus multiple-lesion approaches, with the specific goal of defining the optimal biological effects of therapy.

Dose-limiting toxicities (DLTs)

Although the level of inter-individual variability is high with biotherapies, the current design of first in human trials aims to have clear, dose-related, expected toxicities usually within a timeframe of 3 to 4 weeks (DLT period) [29]. However, because local tissue inflammation/damage generated by intratumoral IT could be problematic beyond 4 weeks, we recommend that investigators and sponsors of HIT-IT trials also take into account adverse events occurring beyond the DLT window (e.g. the 3-month non-healing rate of superficial injected lesions when determining the RP2D of HIT-IT).

Summary conclusions

Recommendations for HIT-IT trial design

The recommendations of the expert panel for HIT-IT are as follows:

1. The patient population should be carefully selected for clinical trials in order to be able to detect clear signs of activity (i.e. patients able to undergo biopsies and with obvious injectable and measurable target tumor lesions).

2. Translational studies should be conducted systematically to facilitate a better understanding of the mechanism of action of the HIT-IT and identify relevant biomarkers of activity.
3. Face-to-face meetings between interventional radiologists, radiologists and/or surgeons and oncologists should be arranged during the screening periods to define and prioritize the radiological assessment of injected and non-injected sites. These discussions and their conclusions should be documented in the patient file.
4. Image-guided injections:
    a. Ultrasound guidance is preferred for the injection of superficial tumor lesions to ensure the correct positioning of the needle
    b. Ultrasound guidance or alternatively CT-scans should be used to guide the injection of deeper tumor lesions
    c. Doppler ultrasound should be used to make sure that no big vessels are injected in error, and thus avoid inadvertent systemic drug/agent delivery.
5. Measurement of injected and non-injected lesions should be performed at each injection time point to better capture the kinetics of tumor growth/response.
6. Diagrams/photographs should be generated at every visit/time point of injection and should be recorded on specific body-map proformas.

Reporting of HIT-IT trial data

Metastatic cancers

The assessment and reporting of the objective response rate (ORR) in injected (enestic) and non-injected (anenestic) tumor lesions is mandatory for the proper evaluation of the efficacy of HIT-IT strategies. The iRECIST criteria, applied separately to injected and non-injected tumor lesions, could be used to evaluate the radiological activity of HIT-IT as they take into consideration the atypical types of responses that immunotherapies can generate (pseudo-progressions, late responses and mixed responses) [30]. However, iRECIST was designed for studying systemic treatments. Conversely, HIT-IT is unique, as tumor responses in both injected and non-injected lesions could be of clinical relevance. It is therefore possible that dedicated tumor response criteria, specific for HIT-IT trials, will eventually outperform and replace the current iRECIST criteria. At the clinical trial population level, the total number of injected versus non-injected tumor lesions should always be documented when providing percentages of responses. Ideally, the median number of injected lesions per patient, the median number of anenestic responding tumor lesions per patient and the median number of total lesions per patient should be reported.

Waterfall plots of HIT-IT trials should be used to report the iRECIST responses of injected and non-injected tumor lesions for every patient, as illustrated in Figure 1. The durations of response for both injected and non-injected lesions should be reported in 3D waterfall plots [31].

- RECIST 1.1 should be used for the overall assessment of tumor responses (as for irradiation, treated tumor lesions should not be considered as target lesions as per RECIST1. 1). Injected and non-injected tumor lesions need to be reported separately.
- For patients with lymphoma, the assessment of the injected versus non-injected lesions should be conducted according to RECIL 17 criteria [32].
- Six-month disease control rate (DCR), duration of response, and the survival of responders may be used instead of ORR to assess clinical efficacy.
- RANO criteria for brain lesions are recommended [33, 34].

Also, the ORR per type of injected lesion should be specified (skin versus subcutaneous versus lymph nodes versus liver versus lung versus other organs).

When systemic therapies are used in combination with intratumoral IT (e.g. intravenous anti-PD-L1), randomization or a strong historical control is required to demonstrate the added value of intratumoral IT on the ORR of anenestic lesions.

In order to assess the ability of HIT-IT to address metastatic disease of poor prognosis, the ORR per type of anenestic lesion should ideally be reported routinely for the following sites:

- ORR of anenestic lung metastases
- ORR of anenestic liver metastases
- ORR of anenestic brain metastases.

In order to assess the impact of priming sites, the ORR of anenestic lesions per type of enestic priming site should ideally be reported routinely for the following sites:

- ORR of enestic skin lesion
- ORR of enestic lymph node lesion
- ORR of enestic liver lesion
- ORR of enestic lung lesion.

Local cancers

Evaluation of the efficacy of a HIT-IT strategy in the localised cancer setting could involve the use of:

- iRECIST ORR or DCR of injected tumor lesions
- Pathological complete response (pCR) rate on surgical specimens
- Relapse-free survival for surgically removed local tumors
- Progression-free survival for inoperable tumors.

The challenges

The major current challenges for the practical implementation of HIT-IT strategies are as follows:

Injectability

All tumor sites are potentially injectable, but the injection of some is more complicated than others. For example, liver metastases that are not visible without contrast-enhanced imaging may be particularly challenging.

Types of injections

The local trauma generated by the needle might have an impact on the local inflammatory response and could be contributing/deleterious to the injected therapy.

Local diffusion

Depending on the tumor type, the interstitial pressure, the level of intratumoral vascularization and necrosis, the local diffusion of injected agents might be non-uniform.

Systemic exposure

Depending on the nature of the drug used, the reversibility of its binding to its target and the vascularization or fat content of the injected tumor lesions, the systemic exposure and PK of the agent might also be variable.

Local combinations

Local combination of immunostimulatory agents raises questions about the stability of the agents when mixed together either *ex vivo* or *in vivo*.

Figure 2 summarizes for investigators and pharmaceutical companies the important points to consider when designing an intratumoral immunotherapy clinical trial.

Key message

The authors consolidated their expertise to provide a series of expert opinions which can be used to provide guidance to clinical investigators, pharmaceutical companies, ethics committees, independent review boards and regulatory agencies when working on or reviewing HIT-IT clinical research, with a view to ensuring the collection of meaningful data from such trials.

Acknowledgements

The authors would like to thank Kate Kronig, Carolina Dalmo, Simona Tettamanti and Klizia Marinoni from the ESMO staff and Sabrine Domiquin from Gustave Roussy for their logistical help in organizing the meeting. Anne Kinsella PhD, of Cancer Communications and Consultancy Ltd, Knutsford, Cheshire, UK is acknowledged for her assistance in the preparation of the manuscript, funded by ESMO.

Funding

The meeting was organized by ESMO. Financial support was provided from the following sponsors: Aduro, Amgen Europe, Bioncotech, Eisai, Idera, Lytix, Merck US (MSD), Nektar, Pfizer, and Roche.

Disclosure

AM reports, fees for Advisory Boards from Merck Serono, eTheRNA, Lytix BioPharma, Kyowa Kirin Pharma, Novartis, BMS, Symphogen, Genmab, Amgen, Biothera, Nektar, GSK, Oncosec, Pfizer, Seattle Genetics, Astra Zeneca/Medimmune, Servier, Gritstone, fees for consultancy from Roche, Pierre Fabre, Onxeo, EISAI, Bayer, Genticel, Rigontec, Daichii Sankyo, Imaxio, Sanofi/BioNTech, research funding from BMS, Merus and Sanofi, being a co-founder of PEGASCY SAS and having a patent for antibodies to CD81. AT is an employee of Merck and Co., Inc USA and declares stocks and shares in Merck and Co., Inc USA. CM is an employee of Pfizer Inc., USA and declares stocks and shares in Novartis AG and Pfizer Inc. CR declares fees for participation in advisory boards for BMS, MSD, Novartis, MERCK, Roche and Incyte. DT is an employee of Bioncotech Therapeutics, Madrid, Spain. EK is an employee of Lytix BioPharma AS, Oslo Norway and declares stocks and shares in Lytix. IM reports fees for advisory boards from BMS, Roche-Genentech, AstraZeneca, Merck Serono, Genmab, Seattle Genetics, F-Star, Bioncotech, Alligator, and Research Funding from BMS, Roche and Alligator. IT is an employee of Esai, Hatfield, UK; JB reports fees for consultancy and advisory roles with Merck and Co, Bayer and Amgen and research funding from Merck and Co, Genetech, Acerta and Celldex Therapeutics. J-FB reports fees for consultancy and advisory roles with Novartis, BMS, MSD and Amgen and research funding from Amgen and MSD. JT reports consulting or advisory roles for Amgen, Bayer, Boehringer Ingelheim, Celgene, Chugai, Lilly, MSD, Merck Serono, Novartis, Pfizer, Roche, Sanofi, Symphogen, Taiho, and Takeda. KH reports fees from consultancy and advisory roles for Amgen, AstraZeneca, BMS, Merck/EMD-Serono, MSD, Oncolytics Biotech, Pfizer, Viralytics and Vyriad and research funding from AstraZeneca, MSD, Oncolytics Biotech and Viralytics. KO reports employment with Amgen Europe GmbH and is a stockholder in Amgen. MI declares employment with Nektar Therapeutics and stocks and shares in Nektar Therapeutics. MH reports employment with Aduro Biotech Europe and stocks and shares in Aduro Biotech Europe. MS reports employment with Roche and stocks and shares in Roche. PA reports fees for consultancy and advisory roles for BMS, Roche-Genetech, MSD, Array, Novartis, Amgen, Merck Serono, Pierre Fabre, Incyte, Genmab, Newlink Genetics, Medimmune, AstraZeneca and Syndax and research funding from

BMS, Roche-Genetech and Array. RL reports fess for consultancy and advisory roles for Merck and Regeneron and employment with Providence Health Services, Earle A Chiles Research Institute, Portland, USA. RA declares fees for consultancy or advisory roles from Novartis, Merck and Aduro and research funding to his institute (the Huntsman Cancer Institute) from Novartis, Amgen, Viralytics, Merck, Tahara, Moderna, Provectus, BBMS and X4 Pharma. RK declares employment with MedImmune/AstraZeneca and shares in AstraZeneca. SR declares fees for consultancy with GuidePoint Inc., and employment with Idera Pharmaceuticals Inc. T de B reports fees for consultancy and advisory roles for GE Healthcare, Guerbet, Erumo and Covidien, and research funding from BTG. JH and J-YD declare no conflicts of interest.

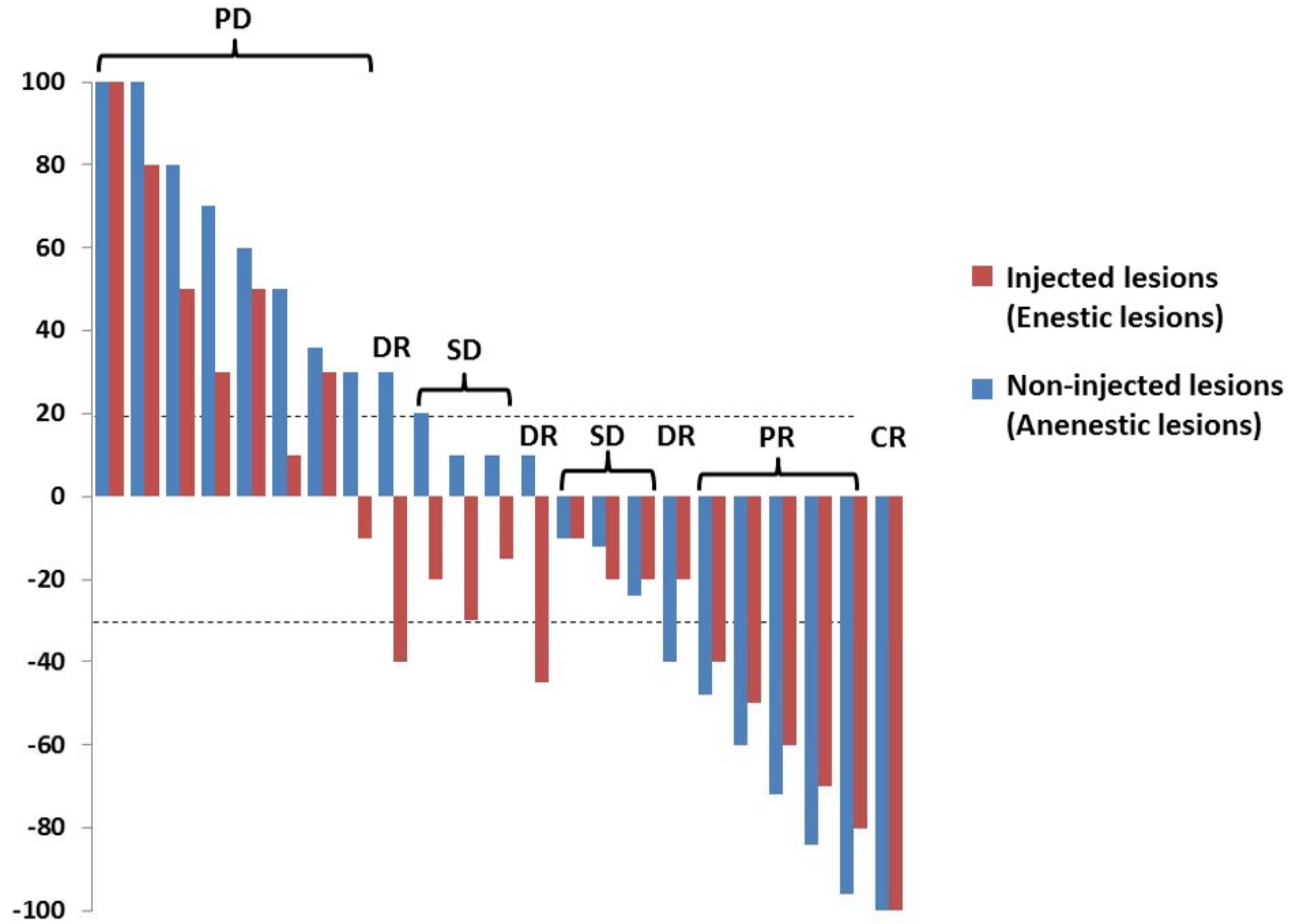
Figure 1. Waterfall plot for HIT-IT trials. Both injected and non-injected lesions should be reported for every patient. Patients should be displayed from progressors to responders according to the ORR of their non-injected lesions. These data are for the purpose of illustration only and are not based on actual clinical data.
CR, complete response; DR, dissociated response; PR, partial response; SD, stable disease

Figure 2: Points to consider when designing an intratumoral immunotherapy clinical trial. ADAs: Anti-Drug Antibodies, PK: Pharmacokinetics; PD: Pharmacodynamics; DLT: Dose-Limiting Toxicities; MTD: Maximum Tolerated Dose; RP2D: Recommended Phase 2 Dose.

References

1. Coley WB. The treatment of malignant tumors by repeated iniculations of erysipelas: with a report of 10 original case. Am J. Medicine 1893; 105: 487-510.
2. Marabelle A, Tselikas L, de Baere T, Houot R. Intratumoral immunotherapy: using the tumor as the remedy. Ann Oncol 2017; 28: xii33-xii43.
3. Brody JD, Ai WZ, Czerwinski DK et al. In situ vaccination with a TLR9 agonist induces systemic lymphoma regression: a phase I/II study. J Clin Oncol 2010; 28: 4324-4332.
4. Marabelle A, Kohrt H, Caux C, Levy R. Intratumoral immunization: a new paradigm for cancer therapy. Clin Cancer Res 2014; 20: 1747-1756.
5. Singh M, Overwijk WW. Intratumoral immunotherapy for melanoma. Cancer Immunol Immunother 2015; 64: 911-921.
6. van den Boorn JG, Hartmann G. Turning tumors into vaccines: co-opting the innate immune system. Immunity 2013; 39: 27-37.
7. Milling L, Zhang Y, Irvine DJ. Delivering safer immunotherapies for cancer. Adv Drug Deliv Rev 2017; 114: 79-101.
8. Aznar MA, Tinari N, Rullan AJ et al. Intratumoral Delivery of Immunotherapy-Act Locally, Think Globally. J Immunol 2017; 198: 31-39.
9. Marin-Acevedo JA, Dholaria B, Soyano AE et al. Next generation of immune checkpoint therapy in cancer: new developments and challenges. J Hematol Oncol 2018; 11: 39.
10. Marin-Acevedo JA, Soyano AE, Dholaria B et al. Cancer immunotherapy beyond immune checkpoint inhibitors. J Hematol Oncol 2018; 11: 8.
11. Mole RH. Whole body irradiation; radiobiology or medicine? Br J Radiol 1953; 26: 234-241.
12. Russell SJ, Barber GN. Oncolytic Viruses as Antigen-Agnostic Cancer Vaccines. Cancer Cell 2018; 33: 599-605.
13. Shekarian T, Valsesia-Wittmann S, Brody J et al. Pattern recognition receptors: immune targets to enhance cancer immunotherapy. Ann Oncol 2017; 28: 1756-1766.
14. Kaufman HL, Kohlhapp FJ, Zloza A. Oncolytic viruses: a new class of immunotherapy drugs. Nat Rev Drug Discov 2015; 14: 642-662.
15. Twumasi-Boateng K, Pettigrew JL, Kwok YYE et al. Oncolytic viruses as engineering platforms for combination immunotherapy. Nat Rev Cancer 2018; epub ahead of print
16. Nestvold J, Wang MY, Camilio KA et al. Oncolytic peptide LTX-315 induces an immune-mediated abscopal effect in a rat sarcoma model. Oncoimmunology 2017; 6: e1338236.
17. Sveinbjornsson B, Camilio KA, Haug BE, Rekdal O. LTX-315: a first-in-class oncolytic peptide that reprograms the tumor microenvironment. Future Med Chem 2017; 9: 1339-1344.
18. Yamazaki T, Pitt JM, Vetizou M et al. The oncolytic peptide LTX-315 overcomes resistance of cancers to immunotherapy with CTLA4 checkpoint blockade. Cell Death Differ 2016; 23: 1004-1015.
19. Zhou H, Forveille S, Sauvat A et al. The oncolytic peptide LTX-315 triggers immunogenic cell death. Cell Death Dis 2016; 7: e2134.
20. Moran AE, Kovacsovics-Bankowski M, Weinberg AD. The TNFRs OX40, 4-1BB, and CD40 as targets for cancer immunotherapy. Curr Opin Immunol 2013; 25: 230-237.
21. Alabanza L, Gnjatic S, Bhardwaj N, Brody J. Intratumoral checkpoint subversion as a strategy for minimizing adverse effects: Harvesting the power of TILs without harvesting TILs. Oncoimmunology 2014; 3: e27580.
22. Dronca RS, Dong H. Immunomodulatory antibody therapy of cancer: the closer, the better. Clin Cancer Res 2015; 21: 944-946.
23. Ando H, Abu Lila AS, Tanaka M et al. Intratumoral Visualization of Oxaliplatin within a Liposomal Formulation Using X-ray Fluorescence Spectrometry. Mol Pharm 2018; 15: 403-409.
24. Bakker RC, van Es RJJ, Rosenberg A et al. Intratumoral injection of radioactive holmium-166 microspheres in recurrent head and neck squamous cell carcinoma: preliminary results of first use. Nucl Med Commun 2018; 39: 213-221.
25. Goins B, Phillips WT, Bao A. Strategies for improving the intratumoral distribution of liposomal drugs in cancer therapy. Expert Opin Drug Deliv 2016; 13: 873-889.
26. Hao Y, Yasmin-Karim S, Moreau M et al. Enhancing radiotherapy for lung cancer using immunoadjuvants delivered in situ from new design radiotherapy biomaterials: a preclinical study. Phys Med Biol 2016; 61: N697-N707.
27. Miller A, Nace R, Ayala-Breton CC et al. Perfusion Pressure Is a Critical Determinant of the Intratumoral Extravasation of Oncolytic Viruses. Mol Ther 2016; 24: 306-317.

28. Tacher V, Le Deley MC, Hollebecque A et al. Factors associated with success of image-guided tumour biopsies: Results from a prospective molecular triage study (MOSCATO-01). Eur J Cancer 2016; 59: 79-89.
29. Postel-Vinay S, Aspeslagh S, Lanoy E et al. Challenges of phase 1 clinical trials evaluating immune checkpoint-targeted antibodies. Ann Oncol 2016; 27: 214-224.
30. Seymour L, Bogaerts J, Perrone A et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. Lancet Oncol 2017; 18: e143-e152.
31. Castanon Alvarez E, Aspeslagh S, Soria JC. 3D waterfall plots: a better graphical representation of tumor response in oncology. Ann Oncol 2017; 28: 454-456.
32. Younes A, Hilden P, Coiffier B et al. International Working Group consensus response evaluation criteria in lymphoma (RECIL 2017). Ann Oncol 2017; 28: 1436-1447.
33. Ellingson BM, Wen PY, Cloughesy TF. Modified Criteria for Radiographic Response Assessment in Glioblastoma Clinical Trials. Neurotherapeutics 2017; 14: 307-320.
34. Okada H, Weller M, Huang R et al. Immunotherapy response assessment in neuro-oncology: a report of the RANO working group. Lancet Oncol 2015; 16: e534-e542.

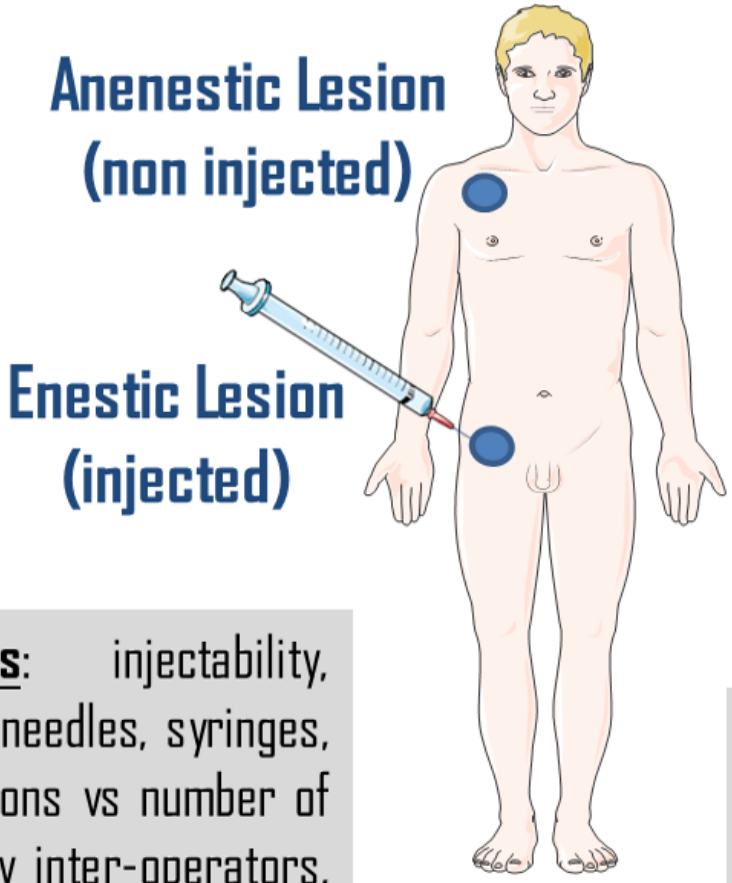

PD
100
80
60
40
20
0
-20
-40
-60
-80
-100
DR
SD
DR
SD
DR
PR
CR
Injected lesions
(Enestic lesions)
Non-injected lesions
(Anenestic lesions)

Dose Determination: per patient vs per lesion, fixed dose/various volumes or fixed concentration,

Trial Design: dose vs drug escalation, lesion escalation vs dose-intensity escalation, priming vs boosting vs prime-boosting,

Efficacy: separate assessment of injected (enestic) and non-injected (anenestic) tumor lesions,

Dose Escalation: DLT definition, DLT period duration, MTD vs optimal dose vs PD read-out for RP2D, bell shape curve effects.



PK : tumor vasculature, volume of lesion vs volume, target expression, reversibility of binding, local metabolism, ADAs, phagocytosis, systemic vs local PK in injected vs non-injected lesions.

Specific issues for oncolytic viruses: local vs systemic replication, distribution, shedding, metabolic vs immune clearance.

Intratumoral Injections: injectability, locations, sizes, guidance, needles, syringes, volumes, number of injections vs number of injected lesions, variability inter-operators, consistence of procedures,

PD: pre-treatment and on-treatment tumor biopsies of injected and non injeted lesions, local and systemic impact of therapy, quantity and quality of the anti-tumor immunity, immune phenotyping in injected and non injected sites, cell recruitment vs cell activation vs cell depletion, timing of events.

Patient Exclusion Criteria: anti-coagulants or significant bleeding diathesis, allergy, risk of vascular catastrophe,