Guidelines for Cancer Immunotherapy

Vol XV

(Part C)

Editors

Dr (Surg Cdr) Gaurav Narula

Dr Jyoti Bajpai

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Tata Memorial Hospital
Dr. Ernest Borges Road, Parel
Mumbai 400 012. INDIA.
Tel.: +91-22-2417 7000
Fax: +91-22-2414 6937
Email: crs@tmc.gov.in
Website: http: //tmc.gov.in

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Dedicated to
all our patients at
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Contributors

Abhishek Mahajan
Akhil Kapoor
Amit Joshi
Arun Chandrasekharan
Gaurav Narula
Hasmukh Jain
Jaya Ghosh
Jyoti Bajpai
Kumar Prabhash
Meenakshi Thakur
Minal Poojary
Rahul Purwar
Rajeev Kumar
Rajesh Bollam
Rushabh Kothari
S V Kane
SD Banavali
Seema Gulia
Subhdeep Bose
Sudeep Gupta
Sujith M
Swaratika Majumdar
Vanita Noronha
Vijay Patil
Vikas Ostwal

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Pankaj Kumar Panda

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Preface

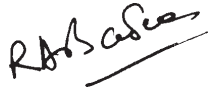
The Evidence Based Management of Cancers meetings conducted by Tata Memorial Centre have been the torchbearers of conscientious use of current best evidence in making decisions about patient care in Indian scenario. At a time when cancer care in India is at the crossroads with rising costs and limited accessibility amongst the general population, it has become imperative to shift focus on greater precision in delivering affordable cancer care. Delivering the right cancer treatment at the right time to the right patient at the right dose and right time will maximize their survival and quality of life. Precision oncology has gained immense clinical significance in the current era and has been making rapid strides moving beyond DNA and exploring other molecular factors that affect tumor behavior. With precise targets for newer therapies in sight, responsible use of this tool will revolutionize the Indian cancer care scenario. It is time for us to steer ahead and give a definitive neoteric direction to precise, evidence based and affordable cancer care.

The theme of the **XVI Evidence Based Management of Cancers in India** meeting 2018 is management of head neck cancers, cancer immunotherapy and pediatric solid tumors. The last three decades of immuno-oncology research have shown that a large number of tumors are recognized by the immune system and their development can be interrupted via immunosurveillance, and immune checkpoints functions are vital for this process. The oncology community witnessed the success of the immune checkpoint inhibition by means of the antibodies targeting the cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed death receptor-1 (PD-1), and its ligand, PD-L1, in treating many historically difficult-to-treat tumor types.Simultaneously, the advent of Chimeric Antigen Receptor- T- cell (CAR) therapy represents a radical departure from all forms of medicine in existence until now and has provided new dimensions to cancer care. Essentially, genetically modified autologous T- cells can be engineered to recognize a tumor antigen and reinfused to the patient as a "live drug" that can potentially clear the identified target in perpetuity. Two such "products" have received USFDA approval in 2017 and many more are expected in 2018.

The promising results of immunotherapies in oncology are generating increasing interest from the oncology community and have the potential to become the "standard of care". However, their huge

costs and the rapid patenting and buy-outs by Industry of technologies developed in academic institutions presents unique challenges to our country. Oncologists of today in India must adapt to rapid changes in practice based on the relevance and applicability of available evidence. Innovative potential also needs to be unleashed within the country and its academic institutions to harness these technologies to keep ultimate costs low, lest the fruits of these developments remain beyond our means for the foreseeable future.

The present EBM Book aims to address the various aspects of immunotherapy from unique mechanism of action to application of the knowledge of immunotherapy in varied indications in our part of the world taking into account the recent advances and practicality of applying them in Indian context. This should serve as ready reference in the clinic for the practicing oncologist in India.



R A Badwe
Director,
Tata Memorial Centre

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CAR T-Cells: Essential Concepts

Gaurav Narula, Rahul Purwar

Background:

T Lymphocytes have long been used in the treatment of advanced malignancies, especially hematological, by the modality of SCT where essentially the donor T-cells exert a Graft versus Leukemia (GVL) effect to eradicate the disease. However, this often comes at the cost of the unwanted Graft versus Host Disease (GVHD), in addition to toxicities due to myeloablative therapy used to prepare the host to receive the graft, and the oft prolonged immunosuppression required post successful engraftment, with its attendant risks of infections and systemic toxicities leading to high TRM and morbidities.

If a patient's own T-cells could be used to overcome the malignancy, this would remove most of the undesirable side effects of SCT. Indeed, the list of tumor antigens recognized by T- cells is long, and rapidly expanding, opening many avenues for potential T-cell therapies.

However, two major biological barriers need to be overcome for this to happen. The patient's T cells are biologically programmed to avoid "own" cells. Secondly, tumor cells, even though antigenically altered, develop several "escape" mechanisms to avoid being targeted by T Cells.

This can be overcome by genetically altering a patients' T Cells to recognize a defined and distinctive tumor antigen and then using them to target the malignancy. This involves removing a patient's lymphocytes by apheresis, segregating the T cells, inserting a gene carrying an anti-malignancy antigen Chimeric Antigen Receptor (CAR) construct using a vector- usually viral- into the T Cells, expanding this genetically altered T Cell population ex-vivo, and then re-injecting them back into the patient so that their own T cells combat their malignancy. Being autologous cells, neither is extensive pre- preparation of the host with toxic therapies required, nor is there any risk of GVHD while retaining the anti-malignancy action like GVL.

Safety Concerns of Genetic Manipulation:

The idea has been around for a while and early efforts focused on the preclinical work and ex-vivo demonstration of efficacy. It is possible that an adverse event of leukemogenesis in a gene therapy trial for Severe Combined Immunodeficiency (SCID) using retroviral vectors delayed human trials using viral vectors for other diseases.

Subsequent research into safety of viral vectors clearly established that insertional mutagenesis for retroviral vectors were probably limited to hematopoietic stem cells and not mature cells, and that lentiviral vectors did not share the same concerns. The NCI group established the protocols for production of clinical grade retroviral vector and validation of their CAR in PBMC of patients with CLL and ALL, and a healthy donor. The efficacy in-vivo in mice has been established by different groups in varying murine models ranging from immunocompetent, to irradiated mice and Immunodeficient mice.

Early Promise:

Chimeric Antigen Receptor (CAR) T-cell therapy has shown promise in multiple relapsed malignancies, especially B-lineage. These genetically altered T-cells recognize a distinctive tumor antigen, the commonest target being CD19, a pan B-cell antigen.

In early trials, “1st generation” CARs with a single cytoplasmic signaling domain from the T-cell receptor derived CD3? chain produced transient results only, due to rapid apoptosis. Adding co-stimulatory domains like CD28 and 4-1BB resulted in “2ndgeneration” CARs with more longevity. Early phase trials in relapsed B-ALL failing multiple therapies had 60- 70% response rates on MRD, with manageable toxicity and translation to long-term survival.

Further modifications with two co-stimulatory domains (3rd generation), and other additions (armored CARs) continue but their advantages are as yet unknown. Focus

is now shifting earlier down the treatment timeline. Industry tie-ups to scale up to Phase III/IV trials are also growing pushing up costs tremendously.

Other developments include refinements in the production of CARs. The earlier trials used retroviral vector, and indeed is still used by two major groups at NCI (National Cancer Institute) and MSKCC (Memorial Sloan Kettering Cancer Center). The University of Pennsylvania (U Penn) group has focused on the lentiviral vector, which may be more efficient. In an early Severe Combined Immunodeficiency (SCID) trial with gene therapy, retroviral vectors were used. Incidents of leukemogenesis were seen in a few patients attributed to triggering of an oncogene close to insertional site in host genome by the retroviral vector. The lentiviral vector however, has a long safety profile, while even with retroviral vectors, the initial concerns have not been borne out in the long term and its oncogenic potential may be limited to hematopoietic stem cells, and not the mature T-cells transfected in CAR generation.

Recently, two CAR T-cell products have been granted USFDA approval for clinical use- Kymriah™ by Novartis®, a CD19 directed CAR T-cell product with lentiviral vector and 41BB co-stimulatory domain, and Yescarta™ by Kite Pharma®, also CD19 directed but made on a retroviral vector with CD28 co-stimulatory domain. While the former is approved for B- ALL cases below 25 years of age, the latter is approved for B Lymphomas in adults. To harness this personalized technology and bring it to the clinic in India requires capacity building of human and capital resources.

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Clinical Grade Car T- Cell Production: What Works and What Doesn't

Minal Poojary, Gaurav Narula

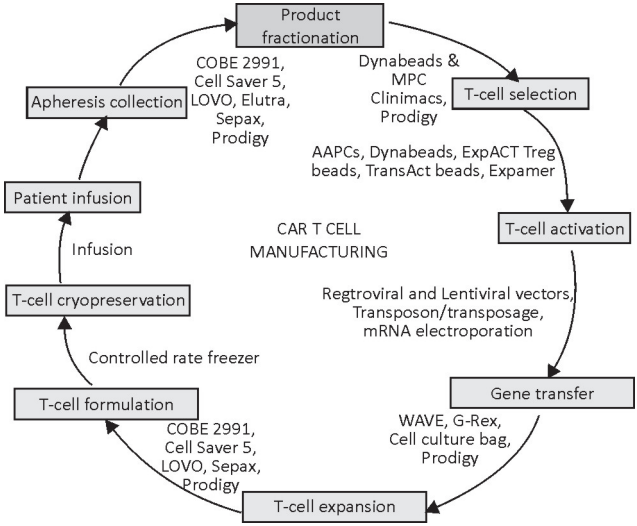
Introduction:

Chimeric antigen receptors (CARs) are genetically modified T cells, which are able to identify and destroy specific malignant cells independent of MHC recognition. They are complex medicinal products with the unique feature of self-amplification and persistence in treated patients. Their translation from basic and pre-clinical research to clinical trials poses many challenges that slow down clinical development. Development of efficient and cost-effective technologies for large scale and reproducible manufacturing under current Good Manufacturing Practices (cGMP) is a prerequisite.

In this chapter, we highlight the production of clinical grade CAR T cells, cGMP manufacturing platforms and the quality control requirements for clinical-grade CAR-T cells in early phase clinical trials.

Production of CAR T cells:

The following process flow provides an outline for production of CAR T cells:



I. Leukapheresis:

- Leukapheresis is most efficient centrifugation method for optimum MNC collection.
- Mature circulating T lymphocytes can be isolated from MNC cell layer located between the dense polymorphonuclear cell/red blood cell (RBC) layers and the less dense platelet layer.
- Several FDA approved apheresis systems are available including COBE Spectra ™ and Spectra Optia from TerumoBCT Inc ® and the Amicus Cell Separator and Com.Tec from Fresenius Kabi Inc. ®.

- T cell yields from these systems vary based on patient, disease and collection factors. Spectra Optia is capable of collecting mature lymphocytes with high efficiency for CAR T cell therapy.
- In patients with advanced malignancy with extensive treatment history, collection of adequate T cells may be difficult.
- Minimum target of 0.6x 10^9 CD3+ cells is required for sufficient transduction and target of 2 x 10^9 CD 3+ cells enables to cryopreserve extra cells in case of subsequent cultures required. Minimum target can be achieved by a good blood draw too. But, leukapharesis gives an added advantage of single draw and storage, if repeat or future CAR T-cells are required.
- 3-6 times the patient's total blood volume should be processed to obtain adequate product.

Points to consider

- Placement of a central venous catheter maintains more consistent blood flow to get high purity products
- Non-mobilized CAR-T cell patients often have low total white blood cell counts, making identification and continued isolation of the RBC-plasma interface challenging (Level of Evidence- 2A).
- Mature lymphocytes are smaller and denser than immature HPCs, which makes removal of RBC contamination more challenging.
- MNC product also contains other contaminants like circulating tumour cells.

II. T cell enrichment:

- The optimal method(s) for T cell enrichment depends on analysis which may be unknown prior to receipt at the manufacturing facility.
- Red cells and platelets can interfere with the flowcytometry lymphocyte enumeration.
- Plasma components may induce clumping in culture, requiring filtration and/or the addition of DNase.
- Monocytes can interfere with the clinical efficacy of some types of therapeutic cells and can also inhibit the CAR-T cell growth.
- So, pre-culture product purification is necessary to reduce the incidence of CAR T cell manufacturing failures.

T cell enrichment can be achieved via a variety of methods-

a) Washing-By closed system, automated cell washers for removal of plasma & other cellular components. Devices such as Haemonetics Cell Saver 5+ ™, COBE2991 ™, and Fresenius Kabi LOVO ™ have the ability to remove gross red blood cells and platelet contaminants.

b) Sedimentating agents-
    a. Density gradients can efficiently remove granulocytes and red blood cell,eg. Ficoll hypaque
    b. There are two approaches to separate monocytes from lymphocytes:
        i. Elutriation- can isolate lymphocytes by counterflow centrifugal elutriation based on size and density.

ii. Antibody bead conjugates- can isolate pure T cell subsets with high specificity via magnetic separation. In addition to selection, it can efficiently provide primary and co-stimulatory signal to expand T cells in culture.

Points to consider

- Ficoll density gradients are incapable of separating lymphocytes from monocytes and may require open systems manipulations (Level of Evidence- 2A).
- Lovo Cell ™ Processing System is a more closed, automated washing system with greater cell recovery (Level of Evidence- 2A).
- Antibody bead technique may mask important cellular epitopes, alter therapeutic cell function and require additional processing to remove the beads. Newly developed biodegradable beads may abrogate the need for such de-beading.
- Contaminations can be reduced by further T cell enrichment but may also result in decrease in T cell yield.

III. T cell activation:

T cell activation is required for ex vivo expansion of T cells and transduction of the CAR cDNA via retroviral vectors. T-cell activation needs a primary specific signal via the T-cell receptor and co-stimulatory signals such as CD28, 4-1BB, or OX40.

Different methods of T cell activation-

a) Cell-based T-cell activation-
   - Dendritic cells (DCs)-Antigen-presenting cells, such DCs, are the endogenous activators of T-cell responses.
   - Artificial antigen-presenting cells (AAPCs)-Cell based AAPCs have been developed where a suitable cell line is modified to express ligands to drive T cell expansion.

b) Beads based T cell activation- Off-the-shelf, clinical grade T-cell activation reagents have simplified the T cell activation process.
   - Antibody-coated magnetic beads: Dynabeads ™ CD3/28 are uniform super-paramagnetic beads covalently coupled to CD3 and CD28antibodies. It enables the selection and activation of T cells in a single step when used in conjunction with the Dynal ClinExVivo ™ MPC magnet.
   - Antibody coated nanobeads- GMP TranAct ™ CD3/28 beads are polymeric nanomatrix conjugated to CD3 or to CD28 monoclonal antibodies and are comparable to Dynabeads ™ CD3/28 for CAR T cell manufacturing.

c) Expamer technology- It can isolate viral-specific lymphocytes by efficiently inducing T cell receptor (TCR) signaling and activating T cells to support retroviral transduction and expansion.

Points to consider

- DC potency varies from patient to patient which limits its usage as a reliable source for T-cell activation (Level of Evidence- 2B).
- The generation and selection of GMP-grade HLA-matched AAPC lines is complex and requires additional resources (Level of Evidence- 2B).
- Removal of the magnetic beads is required at the end of the manufacturing process in most beads based T cell activation (Level of Evidence- 2B).
- TransAct ™ CD3/28 beads are biodegradable although upstream T-cell purification is needed prior to activation (Level of Evidence- 2B).
- Expamer activated T cells can be used for large scale manufacturing as it can be easily added and removed from cell suspensions and provides consistent product purity to enable automation (Level of Evidence- 2B).

IV. Genetic modification of T cells:

Robust CAR gene delivery can be achieved by viral or non-viral gene transfer systems for stable CAR expression. Following are the major types of gene delivery vectors used in clinical expression.

Points to consider

- Viral vectors have the ability to transduce different types of cells with wide tropism with high efficiency.
- There is substantial cell loss during manufacture with all methods of gene modification due to some degree of ex vivo cytotoxicity.

	Viral Vectors			Non-Viral vectors		
Features	Retrovirus	Lentivirus	Adenovirus	Liposomal	Messenger RNA	Transposon/ transposase
Structure	ssRNA	ssRNA	dsDNA			
Infected cell	Dividing cells	Dividing & quiescent cells	Dividing & quiescent cells		Dividing & quiescent cells	
Integration	Yes	Yes	No	No	No	Poor
Advantage	Higher infection rate			Safety, ability to transfer large size gene, less toxicity		
Challenges	Immunogenicity, carcinogenicity, poor target cell specificity, inability to transfer large size genes			Low transfection efficiency, poor transgene expression, mRNA transfection requires repeat dosing in many applications		
Cost of production	Costly and laborious			Cheap and relatively simple		

IV. T cell expansion:

- Several expansion platforms are available to generate therapeutic doses of CAR-T cells.
- Closed culture systems reduce the risk of contamination and facilitate efficient media exchange to promote optimal *ex vivo* expansion.
- Advanced modular components such as gas permeable rapid expansion culture ware allow highly scalable and custom-fit manufacturing which can lead to more rapid and agile development of CAR T cell therapy.

Following are different expansion platforms-

a) Using GE bioreactors- Cellbag bioreactor with a temperature enabling electric rocking base maintains bag inflation & gentle rocking helps in gas transfer and mixing. Its perfusion function allows automatic feeding and waste removal.

b) Using G-Rex bioreactors- It is a cell culture flask that allows cells to grow to high density without compromising gas exchange. Low seeding density, one time upfront feeding regimen, volume reduction feature during harvest are its advantages.

c) Using Clinimacs Prodigy ™- Fully integrated and automated instrument which can accomplish multiple processes like cell washing, magnetic cell separation as well as cell cultivation, an “all-in-one” approach.

Points to consider

- In process cell sampling is not recommended in G-Rex bioreactors as cell disturbance can affect expansion kinetics.
- Advantage of prodigy is its flexible and automated programming and manufacturing procedures.

Quality assessment:

Quality management system should ensure continuous control, traceability, and documentation for all processes including the approval or rejection of the product for release (6). Final product should be released after ensuring the safety, purity, sterility and potency by following assays:

CAR -T CELL RELEASE TESTS

Points to consider

- Capability of entering the relevant cell population is a result of efficient gene delivery system and optimal processing can maximize both the therapeutic dose and in vivo persistence (Level of Evidence- 2B).

CAR-T cell therapy is a personalized medicine which depends on the release of complex biological products. Achieving sufficient number of cells displaying consistent quality at relatively low cost is challenging. The quality of CAR-T cell products largely depends on the manufacturing environment as well as the quality and availability of ancillary raw materials and reagents and also requires careful monitoring and integration into the manufacturing process.

Parameter	Release testing for CAR-T introduced by retroviral or lentiviral vector	Release testing for CAR-T introduced by transposon/ transposase	Release testing for CAR-T introduced by mRNA electroporation
Safety	Gram stain/ sterility	Gram stain/ sterility	Gram stain/ sterility
	Mycoplasma	Mycoplasma	Mycoplasma
	Endotoxin level	Endotoxin level	Endotoxin level
	Copies of transgene expression		
	RCR/RCL		
Purity	% CD3+ T cells	% CD3+ T cells	% CD3+ T cells
	% CAR T cells	% CAR T cells	
	Residual tumor burden	Residual AAPCs	
	Residual beads		
Identity		% CAR T cells	
Potency	In vitro CTL or IFN-gamma secretion		

AAPC- Artificial Antigen Presenting Cells; CTL-Cytotoxic T lymphocyte; IFN-Interferon; RCR- Replication Competent Retrovirus; RCL- Replication Competent Lentivirus

Validation of CAR T cell processes:

For successful production of CAR T cells, proper validation is necessary to ensure that the manufacturing facility is capable of consistently providing a quality clinical grade product. It can be validated in three stages: (1) process design, (2) process qualification and (3) continued process verification.

a) Process design-

CAR T cell manufacturing facilities must validate any process, policy or procedure that has potential impact on the quality of the product and involves the translation of bench developed protocols to clinical grade manufacturing. It requires raw materials and components qualified or approved for human use including GMP grade viral vectors.

b) Process qualification-

It includes design and qualification of infrastructure and equipment. CAR T cell manufacturing involves substantial manipulations and each step requires higher grade of process control and laboratory sophistication compliant with cGMP regulations. Installation and operational qualifications of utilities and equipment must occur prior to obtaining data for validation studies.

c) Continued process verification-

Significant variation between individual products makes this stage challenging. So, continual monitoring is necessary to ensure the process continues to perform as initially validated. Rigorous and regular statistical analysis can aid in identifying significant variation.

To overcome these hurdles, well controlled and rigorously validated manufacturing protocols should be implemented.

Future outlook:

CAR T cell therapy is poised to become widely available, but is still in its early stages of dissemination and transfer to industry-scale manufacturing. Being able to efficiently expand the targeted cell population to therapeutic dose to be released to the patient in a timely manner remains a challenge. Large scale manufacturing, distribution and delivery requires robust and scalable infrastructure both within industry and health care delivery sites.

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Riding the Storm- Cytokine Release Syndrome (CRS) and other Fallouts

Gaurav Narula, SD Banavali

Cytokine Release Syndrome (CRS):

CRS is the major challenge in CAR therapy across models and trial designs, and indeed is a direct result of the biological activity of the therapy. Tumor burden at time of re-infusion, lymphodepletion strategy and the nature of co-stimulatory domains used may all play a role.

Strategies to mitigate tumor burden before CAR T-cell infusion would help reduce its incidence and severity. Levels of cytokines rise rapidly once effective tumor killing begins. Tumor necrosis factor (TNF±) usually rises first, followed by IFN³, IL-1b, IL-2, IL-6, IL-8, and IL-10.

Their release induces a clinical spectrum which can consist of low grade fever in its mildest form, to a rapidly evolving syndrome which includes hectic fevers, rash, hypotension, respiratory distress, coagulopathy, transamnitis, and organ dysfunctions including cardiac, renal shutdown and a spectrum of neurological manifestations ranging from headaches to delirium and seizures.

Management of the syndrome is mostly supportive with a fluid challenge and low dose vasopressor used early in

the management. Patients requiring more support than this and developing features of organ dysfunction are categorized as Grade 3 toxicity and beyond.

While many cytokines are released, and indeed in malignancy patients, baseline levels of some of these too may be higher than in normal subjects, it is the rise of IL-6 which is the most dramatic. Use of IL-6 inhibitor Tocilizumab, can rapidly ameliorate even severe toxicities.

Steroids are effective too, but are deferred for severe toxicity only, or used in short courses to avoid suppressing the infused CAR T-cells and deny the patient of opportunity for them to act.

While all major groups have published their experiences with CRS, and the existence of authoritative review,a cross-institutional group led by Lee DW, has published the guidelines for the management of CRS incorporating the best of the experiences seen by all the groups involved in CAR therapy as described above, and evolving a one-stop resource that has stood the test of time.

B-Cell Aplasia:

The CAR T-cell is programmed to attack all cells carrying the B-cell antigen CD19. This antigen is also carried by normal B-cells in the body, which perform the vital function of immunoglobulin production that plays an important role in host defense against infections, especially from encapsulated bacteria such as meningococcal, pneumococci, H Influenza and staphylococci, and many viral infections in conjunction with T-cells.

The successful eradication of malignant cells by the CAR T-cell also results in B-cell aplasia and the resultant

agammaglobulinemia can make the patient prone to repeated infections, which may be life-threatening. These infections are usually easily treated with available antibiotics if reported early, as resistance in not commonly seen.

Routine monitoring of serum immunoglobulin levels and B-cell counts by flow cytometry, should be done post CAR T-cell infusion

Serum IgG levels below 400 mg/m2 warrant routine IVIg infusion at 3-4 weekly intervals, but can be avoided if patient remains on close follow-up, can report early if febrile and has been asymptomatic for it.

Secondary Malignancies:

By virtue of inserting a gene into the T-cell to express anti-CD19, the host genome of the T-cell is altered. This carries a hypothetical risk for second malignancies in later years. This may occur by inactivation of a tumor-suppressor gene or triggering of an oncogene near the insertional site.

This was seen in one of the earliest gene-therapy trials more than 20 years ago, in some of the patients who received gene therapy for Severe Combined Immunodeficiency (SCID)- a congenital disorder, which is uniformly fatal in the first year of life itself if not treated. It was later found to be related to the viral vector used.

Since then several developments have taken place, which have made viral vectors safer. Extensive tests in many laboratories world-wide, and many more human trials have since shown this risk to be non-existent over extended follow-ups. However, not too many patients have

been followed for very long for us to say there is no such risk. For this reason, all patients receiving genetically modified cells in any form should follow up for up to 20 years to discover any such occurrence.

Management of CRS

Adapted from: Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, et al. Current concepts in the diagnosis and management of cytokine release syndrome. Blood. 2014;124(2):188-95.

Table 1. Cytokine Release Syndrome: Clinical Features

Organ System	Clinical Features
Constitutional	Fever + - Rigors, malaise, Fatigue, Anorexia, Myalgias, Arthralgias, Nausea, Vomiting, Headache
Skin	Rash
Gastro-intestinal	Nausea, Vomiting, Diarrhoea
Respiratory	Tachypnea, Hypoxemia
Cardiovascular	Tachycardia, wide pulse pressure, hypotension, increased cardiac output (early sign), diminished cardia output (late sign)
Coagulation	Elevated D-dimer, Hypofibrinogenemia,
	+ - Bleeding
Renal	Azotemia
Hepatic	Transamnitis, Hyperbilirubinemia
Neurologic	Headache, Mental status changes, Confusion, Delirium, Word finding difficulty or frank aphasia, Hallucinations, Tremors, Dysmetria, Altered Gait, Seizures

Table- 2: Grading System for CRS:

Grade	Toxicity
I	No life-threatening symptoms, and only requiring symptomatic treatment, for eg. Fever, nausea, fatigue, headache, myalgia, malaise
II	Symptoms require, and respond to, moderate intervention, with Oxygen requirement <40%, or Hypotension responsive to fluids or low dose of 1 vasopressor, or Grade 2 organ toxicity
III	Symptoms require, and respond to, agrresive intervention, with Oxygen requirement >40%, or Hypotension requiring high dose of, or multiple vasopressors, or Grade 3 organ toxicity or Grade 4 transamnitis
IV	Life-threatening symptoms Requirement for Ventilator support, or Grade 4 Organ toxicity (excluding transamnitis)
V	Death

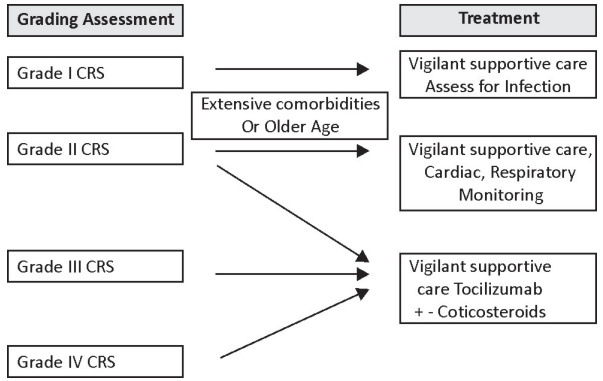

Figure- 1: Treatment Algorithm for CRS:

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Adverse Effects of Immunotherapy

Rushabh Kothari, Jyoti Bajpai

Introduction

Immune Checkpoint inhibitors (ICPi) have revolutionized the treatment of patients with various difficult to treat malignancies and future seems promising for many others. Immune-related adverse events (irAEs) are the common but unique set of adverse effects caused by immune activation seen when these agents are used.

IrAEs include a range of dermatologic, gastrointestinal (GI), endocrine, hepatic and pulmonary toxicities, as well as several other less common inflammatory events .It is of utmost importance that caregivers should be aware of these side effects so as to promptly recognize, identify and manage these irAEs which can otherwise evoke severe or even life-threatening situations.

Multidisciplinary approach should be always considered and the concerned medical specialties should be educated regarding the toxicity profile of immunotherapy and their services should be promptly asked for whenever needed.

The onsets of these adverse events are variable as shown in table 1.

Table1. Variable onset of Immune related adverse events

Adverse effect	Timeline
Dermatologic manifestations	4 to 10 weeks
Hypophisitis	6 week onwards
Diarrhoea/Colitis	3 to 10 week
Episcleritis/Uveitis	8 week onwards
Neurologic syndromes	1 week onwards
Pancreatitis	6 week onwards

Organ Specific Immune related Adverse events (irAEs)

Immune-related skin toxicity

Dermatological irAEs are among the most frequent AEs and have been observed in up to 44% of patients with Ipilimumab. However, less than 2% of them were severe (grade 3 or 4).Maculopapular rash is the most common toxicity and are usually are Grade 1. Vitiligo and pruritus are also seen of which vitiligo is often considered as a marker of response. Grading of skin AEs as per CTCAEv4 is done based on the body surface area involved.

Treatment

- Grade 1 skin AEs: continue treatment and observe for at least 1 week with ICPis along with topical emollients, antihistamines in the case of pruritus and/or topical (mild strength) corticosteroid creams.
- Grade 2 skin AEs: stop ICPis and reinitiateICPi when grade 1.Continue symptomatic treatment as above

- Grade 3 skin AEs: interrupt ICPi and start immediate treatment with topical emollients, antihistamines and high strength corticosteroid creams [level of evidence IIB].Oral steroids (Prednisolone 1mg/kg/day) with PPI cover to be started. Dermatologist reference on individual basis to be considered.
- Grade 4 skin AEs: discontinue ICPi (permanently), consider admitting patient and consult dermatologist immediately. Start i.v. corticosteroids [1–2 mg/kg methyl prednisone], shift to oral prednisolone equivalent and then taper over 4 weeks based on response of AE [level of evidence IIB].

Immune-related endocrinopathies

Thyroid dysfunction, hypophysitis and adrenal insufficiency are common endocrinological AEs seen during treatment with ICPis. Thyroid dysfunction is most common upon treatment with anti-PD-1/PD-L1 (5-10%) or combination of anti-CTLA4 and agents blocking the PD-1/PD-L1 axis (10-20%). Hypophysitis is more common with high dose anti–CTLA4 (10-15%) and its combination with PD-1 blockers (5-10%). Clinical signs and symptoms may be vague like fatigue, myalgia, headache, anorexia but some patients may also have new onset atrial fibrillation, amenorrhea, visual defects, hyponatremia etc. High index of clinical suspicion is important. Immune-related endocrinopathies are usually detected after at least 6 weeks of treatment and they may take months to resolve or may even be irreversible. Adrenal insufficiency due to ACTH deficiency appears to be the earliest change found in patient with hypophysitis.

Treatment

- All patients should have electrolytes, cortisol, glucose, TSH and free T4 at baseline and prior to each cycle of immunotherapy. If these values are abnormal and there is clinical suspicion of hypophysiits, additional pituitary profile which includes ACTH, LH, and FSH, prolactin, IGF1, testosterone (men) / oestradiol (women) should be done.
- In the case of hypophysitis, start prednisone 1 mg/kg orally and taper over 2–4 weeks. Alternatively, intravenous hydrocortisone 100 mg TDS can be started and then tapered after shifting to oral steroid equivalent. Start HRT depending on the affected hormonal axis (levothyroxine, hydrocortisol, testosterone) [level of evidence VB]
- In symptomatic hyperthyrodism patients (grade 1 or 2 tremor, palpitation, insomnia, weight loss etc), interrupt ICPi, start beta-blocker therapy (propranolol or atenolol/metoprolol). Restart ICPi when asymptomatic [level of evidence IV-VB]. Endocrinology opinion is also to be taken.
- In the case of hypothyroidism after excluding cortisol insufficiency, start HRT depending on the severity (50–100 ug/day). Increase the dose until TSH is normal. Endocrinology opinion for long term follow up and dosing can be sought. Consider interruption of ICPi treatment when symptomatic [level of evidence IV-VB].

Gastrointestinal toxicity

Anti CTLA4 agents like Ipilimumab have been known to cause GI toxicity which is more in frequency and severity when compared to PD-1 inhibitors. Diarrhea which usually develops around 6-7 weeks is seen in nearly 27-54% with Ipilimumab and colitis in about 8-22%.Colonic perforations are seen in 1-1.5% treated with Ipilimumab whereas with Nivolumab, there is usually only mild diarrhea. Combination immunotherapy is more toxic than either alone. GI toxicity is commonly the first reason to stop Ipilimumab therapy and hence needs to be differentiated from infectious causes of diarrhea. Stool microscopy, cultures and Clostridium difficile testing should be considered in all patients with Grade 2 or above diarrhea.

Treatment

- Grade 1 diarrhea: In patients with non-severe diarrhoea (grade 1), ICPi can be continued. Treatment with antidiarrhoeal medication (e.g. loperamide) should be prescribed. Loperamide 4mg PO stat dose followed by 2mg after each loose stool or every 2 hours to a maximum 16mg daily can be given. Reassess at 24 hours to ensure that symptoms are not progressing. If symptoms does not improve by 5-7 days or there is any abdominal cramps, stop loperamide and treat as Grade 2 [level of evidence IV-VB].If Grade 1 diarrhea with blood per rectum, fever, cramps, high WBC count, nausea, low albumin etc, high suspicion for severe colitis needs to be kept.
- Grade 2 diarrhea: assess for other toxicities. If present, consider hospitalization and iv hydration. If none, oral

hydration to be encouraged. ICPi should be interrupted and the patient should start with corticosteroids depending on the severity and other symptoms (either budesonide or oral corticosteroids 1 mg/kg/day, max 60mg/day prednisolone with PPI cover).In case of improvement, continue steroids at same dose for minimum 1 week or till symptoms resolve and then taper over 4-6 weeks. Prophylactic antibiotics for opportunistic infections to be started. In the case of no improvement within 3–5 days treat as Grade 3 under hospital admission. Colonoscopy should be carried out and, in the case of colitis, infliximab 5 mg/kg should be administered [level of evidence IV-VB] which can be continued 2 weekly.

- Grade 3 /Grade 4 diarrhea: In these patients with severe diarrhea, permanently discontinue ICPi. Admit patient to the hospital and initiate methylprednisone 2 mg/kg i.v. Gastroenterology consultation to be considered. Abdominal X ray/ CT to rule out perforation is to be done. Close monitoring of clinical signs, fluid balance, diet and stool output is necessary. Dietician reference if needed should be taken. Regardless of improvement, maintain initial steroid dose for e”1 week and then on discharge, change to oral steroids and taper over 3-6 weeks. If no improvement after 3 days of intravenous steroid, evaluation for GI perforation or peritonitis to be considered. Repeat endoscopy by Gastroenterology team to be done if persistent symptoms or clinical deterioration. If concomitant hepatitis, Mycophenolate Mofetil to be added. Infliximab 5mg/

kg at 2 weekly intervals can be useful when steroids fail.

Immune-related hepatotoxicity

Liver toxicity has been reported in about 5-10% of the patients on single agent and 25-30% (of which about 15% is grade 3) in combination immunotherapy and usually appears after 6 weeks of treatment and consists of liver enzymes and bilirubin elevations or even acute hepatitis.

Treatment

- Grade 1 hepatitis: monitor LFT weekly, exclude other causes of liver injury
- Grade 2 hepatitis: withhold ICPi and monitor AST/ALT levels closely (1–2 times/week). When no improvement over 1 week, start oral prednisone (0.5–1 mg/kg). Taper over several weeks under close monitoring of AST/ALT and bilirubin [IV–V, B].Resume ICPiwhen AST & ALT <3x ULN & bilirubin < 1.5x ULN.
- Grade 3 hepatitis: discontinue ICPi and immediately start with intravenous (methyl) prednisone 1–2 mg/kg. When no improvement in 2–3 days, add MMF (1000mg 3_ daily). Taper immunosuppression over 4–6 weeks under close monitoring of AST/ALT and bilirubin [level of evidence IV-VB]
- Grade 4 hepatitis: permanently discontinue ICPi, admit patient to the hospital and initiate (methyl)prednisolone 2 mg/kg i.v. Add MMF if no improvement is observed within 2–3 days. Consult hepatologist if no improvement under double

immunosuppression. Other immunosuppressive drugs to consider are ATG and tacrolimus. Consult or refer patient to an experienced centre. Taper over 6 weeks under close monitoring of liver tests [level of evidence IV-VB].

Immune-related pneumonitis

More commonly seen with anti-PD-1/PD-L1 antibodies than antiCTLA-4 antibodies, the onset, clinical and radiological picture is variable. The incidence is around 1-5% and in case of clinical suspicion, high resolution CT chest is indicated. Acute interstitial pneumonitis/Diffuse alveolar damage syndrome is the most acute life threatening event.

Treatment

- Grade 1 pneumonitis: continue therapy and monitor : if worsens treat as Grade 2
- Grade 2 pneumonitis: interrupt ICPi therapy, try to rule out infection and start with prednisone 1–2 mg/kg orally. Taper over 4–6 weeks [level of evidence IV-VB].
- Grade 3 and 4 pneumonitis, discontinue ICPi permanently, admit the patient to the hospital, even ICU if necessary and immediately start high-dose (methyl)prednisone 2–4 mg/kg i.v. Add infliximab, MMF or cyclophosphamide in the case of deterioration under steroids. Taper over a period of 4–6 weeks [level of evidence IV-VB]. Consider respiratory consultation.

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Response Evaluation of Cancers on Immunotherapy Management

Abhishek Mahajan, Meenakshi Thakur

Introduction

Imaging evaluation of response to traditional cancer treatment with cytotoxic chemotherapy, radiation therapy, or surgical resection is based on a reduction in size of the tumor and the absence of new tumor in accordance with the World Health Organization criteria or the Response Evaluation Criteria in Solid Tumors (RECIST).

Context

Imaging of cytotoxic antitumor agents

- Cytotoxic agents directly kill a tumor cell or prevent tumor cells growth
- Early increase in tumor burden and/or an early increase in tumor size signifies progressive disease
  - Once progression is detected, drug cessation is recommended
- Response after initial treatment of a cytotoxic agent can often predict remission and survival

Imaging of cytostatic antitumor agents

- Lesion regression may not occur to the same extent or magnitude.
- SD should be included in the 'preferred' categories of response.
- Duration of response is important——A total (or significant) reduction in tumour bulk is meaningless if the duration is short lived and the disease recurs.

Imaging of immunotherapy agents

- These agents stimulate an innate immune response against the tumor
- RECIST may not provide a complete assessment of immunotherapeutics.
- Two therapeutic classes are currently available and readily identifiable:
- TKIs (identified by the suffix "-nib" on their INN, e.g. gefitinib, erlotinib, afatinib, crizotinib, etc.),
- Monoclonal antibodies targeting transmembrane proteins (identified by the suffix "-mab" on their INN, e.g. cetuximab, bevacizumab, necitumumab or nivolumab)

Immune-related response criteria (irRC)

Although *RECIST* provides a standardized and practical method to assess response and define progression in solid tumors in general, pitfalls and limitations of *RECIST* have been noted in patient receiving immunotherapy. *It is observed that:*

(1) time to response may be longer for immunotherapy;
(2) response may occur after an initial pseudo-progression;
(3) discontinuation of treatment may be inappropriate in case of progressive disease (PD) unless PD is confirmed after at least 4 weeks;
(4) clinically insignificant PD, such as small new lesions in presence of other responsive lesions, should not be considered
(5) durable stable disease may represent antitumour activity.

Overall, irRC are based on three main principles:

- Tumour burden: devalues the importance of each target lesion in favour of the whole 'quantity' of disease.
- Confirmation: any response, other than stable disease, requires to be confirmed by a consecutive assessment at least 4 weeks after first documentation.
- New lesions: do not necessarily represent a PD. They must be included into the whole tumour burden and their significance is subordinate to the following confirmation.

The developers of the irRC based their criteria on the WHO Criteria but modified the approach to measurement of tumor burden and assessment of response.

Measurement of tumour burden

In the irRC, tumour burden is measured by combining 'index' lesions with new lesions. Ordinarily tumour burden

would be measured simply with a limited number of 'index' lesions (that is, the largest identifiable lesions) at baseline, with new lesions identified at subsequent timepoints counting as 'Progressive Disease'. In the irRC, by contrast, new lesions are simply a change in tumour burden. The irRC retained the bidirectional measurement of lesions that had originally been laid down in the WHO Criteria.

Assessment of immune-related response

In the irRC, an immune-related Complete Response (irCR) is the disappearance of all lesions, measured or unmeasured, and no new lesions; an immune-related Partial Response (irPR) is a 50% drop in tumour burden from baseline as defined by the irRC; and immune-related Progressive Disease (irPD) is a 25% increase in tumour burden from the lowest level recorded. Everything else is considered immune-related Stable Disease (irSD). The thinking here is that even if tumour burden is rising, the immune system is likely to 'kick in' some months after first dosing and lead to an eventual decline in tumour burden for many patients. The 25% threshold allows this apparent delay to be accounted for.

Immune-related response patterns:

- Pattern A: response in baseline lesions evident by 12 weeks since the initiation of therapy, with no new lesions
- Pattern B: "stable disease," which in some patients is followed by a slow steady decline in total tumor burden

- Pattern C: responses after an initial increase in total tumor burden
- Pattern D: a reduction in total tumor burden during or after the appearance of new lesions at time points later than 12 weeks since the initiation of therapy

Comparison of RECIST and irRC

	RECIST	**irRC**
New, measurable lesions (i.e. ≥5 x 5 mm)	Always represent PD	Incorporated into tumor burden
New, nonmeasurable lesions (i.e. <5 x 5 mm)	Always represent PD	Do not define progression (but preclude irCR)
Non-index lesions	Changes contribute to defining BOR of CR, PR, SD, and PD	Contribute to defining irCR (complete disappearance required)
Complete Response (CR)	Disappearance of all extranodal target lesions. All pathological lymph nodes must have decreased to <10 mm in short axis	Disappearance of all lesions in two consecutive observations not less than 4 weeks apart
Partial Response (PR)	At least a 30% decrease in the SLD of target lesions, taking as reference the baseline sum diameters	≥50% decrease in tumor burden compared with baseline in two observations at least 4 weeks apart

	RECIST	irRC
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD	50% decrease in tumor burden compared with baseline cannot be established nor 25% increase compared with nadir
Progressive Disease (PD)	SLD increased by at least 20% from the smallest value on study (including baseline, if that is the smallest) The SLD must also demonstrate an absolute increase of at least 5 mm (two lesions increasing from 2 mm to 3mm, for example, does not qualify)	At least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 weeks apart
*Total Burden=SPD index lesions + SPD new, measurable lesions		

Immune-related adverse events

Immunotherapy is associated with toxic effects that involve multiple organ systems, including the neurologic, gastrointestinal, renal, hepatic, cardiac, pulmonary, skin, muscular, endocrine, and hematologic systems. It is important for the imager to recognize the unique adverse events associated with immunotherapy to guide appropriate treatment and avoid potential imaging pitfalls

that could be mistaken for metastatic progression of disease.

Pseudoprogression

Represents a risky situation because it may cause treatment to stop. It must be stressed that among patients showing an early pseudoprogression there are many who will later show major responses (complete response + partial response). *Three hypotheses try to explain 'pseudoprogression'.*

(1) Homing of cytotoxix T lymphocytes (CTLs) into the tumour following the treatment. Massive infiltration of the tumour by T lymphocytes is demonstrated after treatment.

(2) Increase of the inflammatory tumour milieu, which may be induced by (re)activated CTL against tumour cells, which in turn can induce a transient enlargement of the tumour mass resulting in a pseudoprogression.

(3) Fast-growing tumour, which may increase its mass up to a clear progression during the interval between treatment initiation and its biological effect: in this case we should tag the effect as 'transient-progression' rather than 'pseudoprogression'.

The three hypotheses also apply to the development of new lesions during the initial phase of treatment.

Weakness

irRC are based on WHO response criteria. The product of the longest perpendicular diameters measures each target lesion. Consequently, tumour burden is the sum of the

products of all the target lesions. It accounts for high interobserver variability, at least in clinical practice. Moreover, measuring tumour burden is time-consuming and it also may represent an issue in clinical practice.

Summary and recommendations

The successful clinical application of cancer immunotherapy has opened a new arena for the treatment of advanced cancers. Cancer immunotherapy is associated with a variety of important radiographic features in the assessments of tumor response and immune-related adverse events. The state-of-the art knowledge of immunotherapy and the related radiologic manifestations are essential for radiologists.

Overall response using the irRC

The overall response according to the irRC is derived from time-point response assessments (based on tumor burden) as follows:

- irCR, complete disappearance of all lesions (whether measurable or not, and no new lesions) confirmation by a repeat, consecutive assessment no less than 4 wk from the date first documented
- irPR, decrease in tumor burden e”50% relative to baseline confirmed by a consecutive assessment at least 4 wk after first documentation
- irSD, not meeting criteria for irCR or irPR, in absence of irPD
- irPD, increase in tumor burden e”25% relative to nadir (minimum recorded tumor burden) confirmation by

a repeat, consecutive assessment no less than 4 wk from the date first documented

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Programmed Death-Ligand 1 Testing: A Potential Biomarker for Immunotherapy

Rajiv Kumar, S V Kane

Introduction

The Food and Drug Administration (FDA) approval of immune checkpoint inhibitors has dramatically changed treatment paradigms for many cancer patients especially those with advanced-stage or metastatic disease. Despite very encouraging progress in the development, many patients fail to respond to checkpoint inhibitors, notably antibodies targeting programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1), and uncertainties remain regarding how best to use these therapies in clinical practice. Given the risk of immune-related and other adverse effects associated with treatment, there is a need to identify biomarkers to predict which patients will or will not benefit. PD-L1 protein expression, as detected by immunohistochemistry (IHC) testing, has been widely used as a predictive biomarker assay for anti–PD-1/PD-L1 therapies. Assay for determination of PD-L1 expression is approved by the US Food and Drug Administration for both

first-line and second-line therapy. However, complex biomarker scenario had emerged for the PDL-1 testing that poses many challenges, which were never faced for personalized medicine. There is no clear understanding among physicians, health care personnel, or patients, regarding which assay to use for PD-L1 testing and whether these various assays are interchangeable because each assay was co-developed with a specific drug therapy (**Table 1**).

Context

Despite these existing controversies to PD-L1 biomarker testing, an increasing demand for PD-LI testing as applications of immune checkpoint inhibition both in different stages and different tumor types inexorably continue to grow. Hence, the current status of PDL1 as potential biomarker need to be addressed and will be discussed as follows:

a) PD-1/PD-L1 EXPRESSION AS A PROGNOSTIC MARKER IN VARIOUS CANCERS

Many studies have indicated that expression of PD-L1 in tumor cells and tumor-infiltrating lymphocytes is correlated with poor prognosis in certain cancers as NSCLC, melanoma, RCC, esophageal and gastric cancers. A meta-analysis conducted by Wang et al in 1157 patients with NSCLC showed that PD-L1 expression was significantly associated with poor differentiation of tumors (poor versus well: odds ratio, 1.91; 95% CI, 1.33–2.75; P = .001) and with worse overall survival (pooled hazard ratio, 1.75; 95% CI, 1.40–2.20; P < .001). In contrast, some studies revealed

that PD-L1 upregulation served as a positive prognostic marker in breast cancer and high-grade serous ovarian carcinoma. This is likely due to an increased T-cell cytotoxic immune response in these cancers. The mechanisms leading to these discrepancies are uncertain and need validation by further studies.

b) PD-1/PD-L1 AS A PREDICTIVE MARKER FOR ANTI–PD-1/PD-L1 TREATMENT

PD-L1 expression has been investigated as a potential predictive biomarker for selecting responders to anti–PD-1/PD-L1 antibody treatment. Current data show that patient outcomes are generally better with these therapies, when there is an increase in PD-L1 expression. A systemic review and meta-analysis from 20 trials including patients with metastatic melanoma, NSCLC, and RCC receiving anti–PD-1/PD-L1 antibodies (4230 metastatic melanoma, 1417 NSCLC, and 312 RCC patients) showed that PD-L1 expression is associated with lower mortality and better clinical response to anti–PD-1/PD-L1 antibodies.

In addition, patients with high vs. low PD-L1 expression were more likely to experience treatment benefit with anti-PD-1/PD-L1 agents (nivolumab, pembrolizumab, durvalumab, atezolizumab, and avelumab) in advanced NSCLC. The use of anti-PD-1/ PD-L1 agents in first-line is now accelerating, driven by PD-L1 IHC biomarker selection as shown in the KEYNOTE 024 study.

However, the place of this biomarker in treatment decisions is still not absolutely clear, because responses are also seen in “biomarker-negative” patients. Most likely this is related, for the most part, to heterogeneous expression in tumors and biopsy sampling error, and to

the fact that PD-L1 expression is a biologic continuum, such that the creation of 'positive' and 'negative' groups defined by a cut-off does not create two distinct categories that each include patients who are equally likely or unlikely to benefit from therapy. Hence, we must keep in mind that there are some limitations to using PD-L1 both as a prognostic as well as predictive biomarker.

c) STRATEGIES TO MEASURE PD-L1/PD-1 EXPRESSION

Assessment of PD-L1 expression through immuno-histochemical staining has been advocated as one potential biomarker. While, its role as a companion or complementary diagnostic assay in the refractory setting has been studied extensively, an even more important role for PD-L1 has emerged for selecting patients for upfront treatment. Different clinical trials have used different IHC assays from different pharmaceutical manufacturers to measure PD-L1 expression. These assays use different monoclonal antibody clones recognizing various epitopes of PD-L1. Various systems for amplification and detection of the signal are used for IHC, leading to different thresholds of detecting PD-L1 expression. Additionally, 3 of these assays evaluate the PD-L1 expression in the tumor cells only. The Ventana SP142 assay, manufactured by Spring Bioscience (Pleasanton, California), measures the PD-L1 expression in both tumor cells and tumor-infiltrating immune cells. Hence, the current "**one drug–one diagnostic test co-development approach"** for the approval of therapeutic products has resulted in the production of individual PD-L1 diagnostic systems for each PD-1/PD-L1 inhibitor as summarized in **Table 1.**

Table 1. Current Programmed Death Ligand-1 (PD-L1) Immunohistochemistry (IHC) Assays with Coupled Treatment Agent

	PD-L1 IHC 28-8 Pharm Dx	PD-L1 22C3 IHC Pharm Dx	Ventana SP142	Ventana SP263
Coupled treatment Agent	Nivolumab (Bristol-Myers Squibb, New York,New York)	Pembrolizumab (Merck, Kenilworth, New Jersey)	Atezolizumab (Roche/ Genentech, San Francisco, California)	Durvalumab (AstraZeneca, London, United Kingdom)
mAb clone	28-8 (Abcam, Cambridge, United Kingdom)	22C3 (Dako, Carpinteria, California)	SP142 (Spring Bioscience, Pleasanton, California)	SP263 (Spring Bioscience)
Diagnostic platform	Dako	Dako	Ventana/Roche (Tucson, Arizona)	Ventana/Roche
FDA status	FDA-approved complementary test for metastatic melanoma and Nonsquamous NSCLC	FDA-approved companion diagnostic test for NSCLC	FDA approved for metastatic uroepithelial cancer	FDA approval for NSCLC

	PD-L1 IHC 28-8 Pharm Dx	PD-L1 22C3 IHC Pharm Dx	Ventana SP142	Ventana SP263
Staining location scored	Membrane	Membrane	Membrane	Membrane
Cell types scored	TCs (Tumor cells)	TCs	TCs and TIIC (Tumor-infiltrating immune cells)	TCs
Cutoff(s) tested	%1, 5%, or 10% of TCs	1%, or 50% of TCs	TCs: 1%, 5%, or 50%;	25% of TCs
			TIICs: 1%, 5%, or 10%	
FDA-approved Thresholds	N/A	50% of TCs	N/A	50% of TCs

This approach had questioned the reliability of PDL1 expression alone as a definitive predictive biomarker for immune check point inhibitors and made the PDL1 testing a challenging task as discussed as follows and summarized in **table 2**.

d) CHALLENGES FOR PDL1 TESTING:

1. ASSAY-SPECIFIC CHALLENGES; CAN A SINGLE PD-L1 IHC TEST FOR ALL ANTI-PD-1/PD-L1 INHIBITORS IS POSSIBLE?

The availability of four PD-L1 diagnostic assays, each individualized for a specific anti-PD-1/PD-L1 agent poses a daunting challenge for patients, clinicians, and other stakeholders seeking access to treatment without overly burdensome diagnostic costs and procedures. It would be ideal for all concerned if there were agreement on a single qualifying test, but this may be impossible to achieve.

In 2015 a workshop by the Food and Drug Administration, the American Association for Cancer Research (AACR), and the American Society of Clinical Oncology led to a Blueprint Proposal developed by 4 pharmaceutical companies (Bristol-Myers Squibb Co, Merck & Co Inc, AstraZeneca PLC, and Genentech Inc), 2 diagnostic companies (Agilent Technologies Inc/Dako Corp and Roche/Ventana Medical Systems Inc), 2 professional societies (AACR–International Association for the Study of Lung Cancer), and 2 regulatory agencies (the European Medicines Agency and the FDA) to evaluate the analytic similarities of the 4 PD-L1 assays for use in NSCLC.

The goal of this effort is to harmonize companion diagnostics for PD-L1 and to assess the possibility of

interchangeable use of these assays. There are 2 phases in this proposal: phase 1 will evaluate analytic components by measuring PD-L1 expression on tumor or immune cells and predefine cutoffs in order to evaluate how these assays would compare using clinical samples; and phase 2 will design a statistically powered study with a large sample size based on the findings of phase 1.

The preliminary results have indicated that 3 antibodies (22C3, 28-8, and SP263) have similar analytic performance in measuring the percentage of PD-L1– expressing tumor cells. A fourth antibody, SP142, constantly labeled fewer tumor cells. However, there is also less agreement between observers when evaluating immune cells compared with cancer cells. Additionally, the patient population defined by Ventana SP263, manufactured by Spring Bioscience, at the 25% cutoff point is similar to the group identified by the Dako 28-8 and Dako 22C3, manufactured by Dako, at the 1% cutoff. However, about 37% of the cases studied revealed discrepant results for PD-L1 expression between assays. While very high and no PD-L1 expression were for the most part concordant among assays, low to moderate expression levels that are seen in the majority of NSCLC patients can result in discrepancy. This suggests the possibility of assignment into different diagnostic categories according to the key clinical cutoffs if assays and algorithms are mismatched.

Although the preliminary phase I Blueprint results are limited in their ability to inform clinical decision making, the phase II portion of this collaborative initiative is ongoing and will hopefully provide more clarity for clinical practice.

Recently some multi-institutional studies had shown a high correlation be-tween PD-L1 IHC expression data obtained with the Agilent PD-L1 IHC 22C3 pharmDx and the Ventana PD-L1 (SP263) tests in NSCLC and suggest that the two assays could be utilized interchangeably as an aid to select patients for first-line and second-line treatment with pembrolizumab and potentially with other anti–PD-1/PD-L1 checkpoint inhibitors. Although challenging, harmonization amongst these various assays is mandatory for immunotherapy in future.

Table 2 Challenges and potential solutions to PD-L1 testing in NSCLC

Challenges	**Potential solutions**
Assay specific challenges	
Inter-assay variability for PD-L1 immunostaining between Dako 28-8, Dako 22C3, Ventana SP142, Ventana SP263	Ongoing cross-industry collaboration, the Blueprint Project, aimed at inter-assay harmonization
Biopsy specific challenges	
Cytology *vs.* histology: (I) intertumoral (primary *vs.* metastatic lesion) heterogeneity; (II) intratumoral heterogeneity	(I) Concordance studies evaluating PD-L1 expression between cytology/histology samples, primary/metastatic lesions; (II) , Automated Quantitative Analysis (AQUA) providing better resolution of PD-L1 expression
Patient specific challenges	
Impact of concurrent *EGFR* mutations and *ALK* rearrangements in PD-L1 positive tumors	Prospective studies looking anti-PD-1/PD-L1 agents in *EGFR* and *ALK* mutated patients

2. BIOPSY-SPECIFIC CHALLENGES

There is growing data to suggest that specific features of a tumor specimen undergoing PD-L1 testing have a profound impact on assay results. While such intertumoral heterogeneity raises questions about choosing the most appropriate site for PD-L1 testing, notable differences in expression even within a tumor are also cause for concern. Therefore, the absence of PD-L1 expression on small biopsies may not reflect the systemic immunologic landscape. This may have contributed to some patients responding to anti–PD-1 or anti–PD-L1 therapy independent of PD-L1 expression. Furthermore, it remains to be clarified whether the PD-L1 expression test should be performed on the primary tumor site or metastatic sites. In one study, discordant PD-L1 expression levels were seen in 14% of cases when paired primary lung and brain metastases were compared. In a separate study of 109 patients with resected stage II and III lung adenocarcinomas, conflicting PD-L1 expression levels between primary tumor and nodal metastases were seen in 38% of cases.

For the most part, PD-L1 testing is performed on histologic specimens. However, recent reports suggest that cytology specimens may provide enough cellularity for some of the assays mentioned. A pilot study revealed that 92% (34 of 37 cases) of cytology specimens had sufficient cellularity for analysis with 22C3 (greater than 100 cells). Challenges related to the false positive expression in the other immune cells in the background of cytology specimen to be addressed by further studies.

3. PATIENT-SPECIFIC CHALLENGES

The expression level of PD-L1 has been reported to be associated with other genetic alternations. The NSCLC cell lines with epidermal growth factor receptor (EGFR) mutations tend to have higher PD-L1 expression on the cell surface. While targeted therapies have become standard of care for these patients, anti-PD-1/PD-L1 therapies have failed to improve upon outcomes further. In a retrospective analysis, patients who harbored these molecular genotypes, the majority of whom (82%) had progressed on prior tyrosine kinase inhibitor therapy, and received treated with anti-PD-1/PD-L1 therapy, had low objective responses rates-3.6% for *EGFR*-mutant and 23% for ALK-positive patients. There are ongoing trials determining the efficacy of combination tyrosine kinase inhibitors with PD-1/PD-L1 inhibitors in this population.

In a phase 2 trial studying pembrolizumab in multiple solid metastatic tumor, patients with mismatch repair–deficient (ie, microsatellite instability–high) colorectal cancer are more likely to benefit from PD-1 blockade (pembrolizomab) than those with mismatch repair–proficient tumors. Interestingly, PD-L1 expression is also elevated in mismatch repair–deficient colorectal cancer patients compared with those with mismatch repair–proficient tumors. However, the PD-L1 expression is not significantly associated with progression-free survival or overall survival.

Further, smoking might have a potential impact on the immune therapy. The higher responses to nivolumab observed in smokers could be explained by this hypothesis,

as tumor mutation burden is high in smokers' tumors Peters et al reported that tumor mutation burden enhanced the predictive power of PD-L1 IHC for selecting patients who benefit from first-line therapy with nivolumab. In contrast, lung cancer patients with EGFR-mutant tumors (known to have low mutation loads) showed lower response rates than those with wild-type tumors, as reported in subset analyses of the nivolumab, pembrolizumab, and atezolizumab trials.

Therefore, therapeutic strategies are essentially different between the tumors of the two compartments. Genetically complex cancer would potentially be a good target for immune checkpoint inhibitors. Genetically less complex tumors are less immunogenic and less responsive to immune checkpoint inhibition, but are generally very responsive to tyrosine kinase inhibitors targeting their oncogenic drivers. Hence, mutation burden in the tumor has been proposed as a predictive biomarker for immune checkpoint inhibitor therapy.

d) PDL-1 IMMUNOHISTOCHEMISTRY REPORTING- HOW TO DO IT?

In practice, the name of the diagnostic kit and the diagnostic criteria of the assay used should be reported. In cases where PD-L1 staining is absent in the tumor, the adequacy of the PD-L1 control section staining should be mentioned. Positive PD-L1 staining is defined as complete circumferential or partial linear plasma membrane staining of tumor cells at any intensity. Nonmalignant cells and immune cells, such as infiltrating lymphocytes or macrophages, and necrosis may also stain positively for

PD-L1; however, these cells should not be included in the scoring for the determination of PD-L1 positivity of tumor cells. In the assay using the SP142 antibody clone, the PD-L1-positive immune cells, as well as the tumor cells, are considered in the criteria of positive PD-L1 staining (Table 1).

Because therapeutic response of immune checkpoint inhibitors is reported to be in proportion to the extent of PD-L1 reactivity, reporting of the extent of positive tumor cells, at least in 10% increments, is recommended. If the immunotherapeutic agent to be used is known at the time of testing, the results can be reported in terms of broader categories (eg, <1%, 1% to 49%, >25/50%), appropriate for the drug to be used.

Recommendation For Pdl-1 Testing As Potential Biomarker For Immunotherapy

i. The emergence of immune checkpoint inhibitors has ushered in dramatic yet exciting progress in oncology practice.

ii. To date, PD-L1 immunohistochemistry (IHC) remains the best validated biomarker for predicting clinical benefit from anti-PD-1/PD-L1 therapies, as demonstrated in many clinical trials especially in lung cancer, melanoma and urothelial cancer(Level I/II evidence) .

iii. The application of the PD-L1 IHC assays is expanding from the current use of some immune check-point monotherapies, mainly in second- or greater-line therapy to first line therapy as well as combination therapy.

iv. Several challenges still remain regarding standardization of IHC beyond the Blueprint study. Questions regarding the biology of PD-L1 expression, including heterogeneity, correlations with stage of disease, ethnical associations, demographic characteristics, impact of prior lines of therapy, and associated co-medications are still remain to be addressed.

v. Variability in the methods used to determine PD-L1 expression suggests a need for standardized use of well-validated PD-L1 diagnostic assays. Results of the Blue print study will possibly give solution to this issue and bring harmonization amongst various available assays.

vi. It remains to be seen whether PD-L1 IHC will be replaced by mutational burden or tumor inflammation assessment, or some other biomarker strategy or, perhaps more likely, the predictive power of PD-L1 IHC may be enhanced by the addition of another test for immunotherapy.

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CAR T Cells in the Management of Acute Lymphoblastic Leukemias

Gaurav Narula, SD Banavali

Introduction:

Currently, Overall Survival (OS) of Pediatric B-Acute Lymphoblastic Leukemia (ALL) in India is around 70%, but above 95% in the best centers abroad with collaborative risk-stratified approaches that improved outcomes and reduced treatment related mortality (TRM) and toxicities. However, 20- 25% ALL patients relapse making it par with Soft Tissue Sarcomas as the 4th commonest pediatric malignancy. Current standard of care is achieving 2nd Complete Remission (CR2) followed by Allogenic Stem Cell Transplant (Allo-SCT), involving several fold increased finances, an HLA matched donor, and attendant high TRM. Additionally, early relapse and persistent Minimal Residual Disease (MRD) have significantly poorer outcome despite Allo-SCT. Most patients, already exhausted of all resources, simply choose palliation.

B- ALL has been one of the highly curable malignancies, especially in children for several years now, with significant

strides made in each decade. The outcome of the same disease in adults, however, has remained dismal. Recently, with the increased use of more aggressive "pediatric like" protocols especially in adolescents and young adults (AYAs), the prognosis has improved somewhat. However, 20- 25% of pediatric ALL and nearly 30- 45% Adolescent and Young Adult (AYA) adult B- ALL cases ultimately relapse. Being one of the commonest malignancies in children, the relapse burden is also commensurately high, making ALL relapse the 4thcommon pediatric malignancy. Another challenge is posed by refractory ALL. With the increasing use of MRD evaluation early in the treatment of ALL, typically at the end of Induction or the first month of treatment, risk of relapse can be differentiated quite easily. MRD above 0.01 % predicts EFS of 30- 59%, while those who are negative (<0.01%) have more than 88% EFS.

Treatment of relapse/ refractory ALL is difficult as it involves intensive chemotherapy ideally followed by allogenic stem cell transplant (Allo-SCT). The latter however, is a capital and human resource intensive procedure, and most crucially requires a matched donor, preferably a sibling (Matched Sibling Donor- MSD), with the same Human Leucocyte Antigen (HLA) signature in at least 10 loci of the HLA domain. Being somatically inherited from both parents, the chances of two siblings being HLA matched is less than 25% on an average depending on family size and ethnic/ familial cloistering. Matched Unrelated Donor (MUD) SCTs are several folds more expensive than MSD SCTs. Most donor registries are in developed nations and of differing ethnicities resulting in lower chances of matching in Indian patients. Moreover, the timing of the

relapse with respect to disease free interval affect the chances of a successful Allo-SCT. Early medullary relapses defined as occurring within 36 months of first remission (CR1) have only 20- 40% chance of successful outcome, while those relapsing in extramedullary sites, or beyond 36 months fare better with 60- 70% chance of successful outcome. An added criterion is the need for a patient to be in remission again (CR2) with MRD negative status prior to SCT. This involves intensive chemotherapy to achieve CR2 and then sustaining this remission till such time SCT is carried out. As number of centers doing Allo SCTs are still few when compared to the need, this often proves challenging and extremely toxic for the patients. Allo-SCT itself carries significant morbidity and TRM, and many patients may suffer long term complications especially GVHD and infections related to long term immunosuppression, and adverse events may actually increase over time. Unlike developed nations, in India the expenses and technical difficulties involved result in less than 10% of patients who might benefit from an SCT opting for one, and only a fraction making it through the entire process.

Trials in CAR T-cells for ALL:

CAR T-cell technology has been used extensively in Phase I & II trials across the globe in relapsed/ refractory setting mostly against B cell malignancies with an Anti- CD19 CAR construct. CD19 is a pan B-cell antigen which is also regularly expressed on most malignant cells of B-lineage including the vast majority of ALL, Chronic Lymphocytic Leukemias (CLL) and B Non-Hodgkin Lymphomas (B- NHLs),

and increasingly recognized as a good target for immunotherapy.

CAR Generations:

- In early trials "first generation" CARs were used mostly with transient results only. These CARs contained a single cytoplasmic signaling domain derived most commonly from the T Cell Receptor (TCR)-derived CD3? chain, which mediated a primary activation signal upon encounter with the targeted Ag (signal 1). However, these would soon undergo apoptosis or develop anergy. (Level of Evidence- 2A)
- Various groups started adding co- stimulatory domains of cytoplasmic signaling (signal 2). CD28 and 4-1BB have been the commonest ones used. The addition of these domains resulted in "second-generation" CARs resulting in more sustained action. Among the two, the 41BB co-stimulatory domain has been seen to confer greater longevity to the CAR-T cell in-vivo. (Level of Evidence- 2B)
- The addition of more than one co-stimulatory domain has led to the formation of "third generation" CARs, which are now being extensively tested by several groups. The second-generation CARs, however, have remained the favored model from pre-clinical and early phase clinical trials, being more stable and capable of sustained activity in vivo. (Level of Evidence- 2A)
- Further modifications continue. These include the addition of cytokines to the CAR, which can modulate

the inhibitory effect of the tumor micro- environment on the CAR, such as the addition of IL- 12, effectively a Signal- 3, resulting in an "Armored CAR" with enhanced local potency.

- Still further modifications include the addition of a "suicide gene" which allows targeted killing of the CAR itself if it has resulted in severe or life- threatening side effects, or has simply outlived its purpose. This can be done by making the T cell present a unique antigen like Epidermal Growth Factor Receptor, which can then be targeted by a known antagonist like the EGFR Inhibitor Cetuximab. (Level of Evidence- 3B)
- Results in early phase clinical trials have also improved with higher generation of CARs and early studies now report 66% to 90% complete response rates. (Level of Evidence- 2A)
- These results are all the more remarkable as most of the patients eligible for early trials have failed several lines of chemotherapy, including SCT in some, and had less than 5% chances of survival by known literature.
- Fueled by these dramatic responses, several groups all over the world have now shifted focus to CARs as the favored approach for improving outcomes in previously known fatal situations, and exploring the utility in earlier settings of the treatment timeline.
- The thrust has been on the treatment of CD19+ ALL Relapses, and refractory CD19+ ALL, CLL and NHLs (9, 19). Newer targets being explored include other targets for B- ALL, AML, Carcinoma Ovary and other malignancies.

Vectors:

Other developments include refinements in the production of CARs.

- The earlier trials used retroviral vector, and indeed is still used by two major groups at NCI (National Cancer Institute) and MSKCC (Memorial Sloan Kettering Cancer Center).
- The University of Pennsylvania (U Penn) group has focused on the lentiviral vector, which may be more efficient.
- In an early Severe Combined Immunodeficiency (SCID) trial with gene therapy, retroviral vectors were used. Incidents of leukemogenesis were seen in a few patients attributed to triggering of an oncogene close to insertional site in host genome by the retroviral vector.
- The lentiviral vector however, has a long safety profile, while even with retroviral vectors, the initial concerns have not been borne out in the long term and its oncogenic potential may be limited to hematopoietic stem cells, and not the mature T-cells transfected in CAR generation. (Level of Evidence- 2A)

Early Trials:

Initial experience with CAR T-cell therapy was limited to case reports with encouraging results using CAR T-cell therapy in relapsed refractory B cell ALL.

- Stephan Grupp et al, from the Children's Hospital of Philadelphia and University of Pennsylvania reported

their experience with the first two patients treated on a pilot study of CAR T cell therapy in pediatric relapsed refractory B cell ALL. Two children with relapsed refractory Pre-B cell ALL, one of whom had relapsed post allogeneic stem cell transplant, were treated with T cells transduced using a lentiviral vector with anti-CD19 antibody (CTL019 cells) and a CD137 (4-1BB) co-stimulatory domain at a dose of 1.4x 106 to 1.2 x 107 CTL019 cells per kg body weight. Key initial observations were that in both the patients the CTL019 cells expanded to more than 1000 times of their initial engraftment level, the cells were identified in the peripheral blood, marrow as well as in the cerebrospinal fluid and persisted for atleast 6 months. Complete remission was seen in both the patients and both were negative for minimal residual disease by the end of one month. The first patient maintained remission at 11 months after treatment, the second patient had a relapse with a CD19 negative blast population at 2 months. A cytokine release syndrome and B cell aplasia were noted in both the patients. MRD negative remission in such an aggressive disease with dismal outcomes provided a proof of principle for its efficacy and further impetus to its use. (Level of Evidence- 4)

- In the series published by Brentjens et.al, from the Memorial Sloan-Kettering cancer center, five adults with relapsed B cell ALL, not previously treated with allo-SCT were given 1.5-3 x 106 autologous 19-28z+T-cells/ kg following prior conditioning therapy with 1.5-3.0gm/ m2 cyclophosphamide. Difference from the

pediatric study was that these patients underwent subsequent allo-SCT. All 5 patients attained MRD negative status, 4 of them proceeded to allo-SCT. One patient could not undergo allo-SCT or receive further CAR T-cells due to co-morbidities and relapsed 90 days post infusion. He had a recurrence with CD19+ blasts suspected to be due to the high doses of steroids he received during the cytokine release syndrome. Assessment of the durability of MRD-responses was limited by the fact that they underwent allo-SCT. Unlike the experience with 4-1BB CAR T-cells, cytokine release syndrome manifested earlier, around 3-5 days and correlated with disease burden. The duration of B-cell aplasia also was shorter compared to the 4-1BB CAR T-cells. So, in relapsed adult ALL, which has worse outcomes as compared to relapsed pediatric B cell ALL, they were able to induce a MRD negative remission with less toxicities and enable them to undergo allo- SCT. (Level of Evidence- 3B)

- Davila et al from the same group, subsequently reported on an expanded cohort with 16 adult patients of relapsed/ refractory B-cell ALL, including the first 5 patients. The median age of the cohort was 50 years, the CAR T-cell dose was 3x106 CAR T-cells/ kg. This was a particularly poor prognosis population as substantiated by the fact that 11/16 had unfavorable cytogenetics, 7 patients had received more than one salvage regimen, and four had been transplanted already. 14 out of the 16 patients were refractory to the last salvage regimen used and 8 patients had morphologically residual disease. Post

CAR T-cell therapy the complete response rate was 88%. MRD negative remission was seen in 75% of patients. (Level of Evidence- 3B)

- They also defined sCRS as a triad of fever persisting for > 3 days, elevation in cytokines by more than 250-fold from baseline and evidence of hypoxia, hypotension or renal failure. C- reactive protein was identified as an important predictive marker to identify sCRS. Tocilizumab was found to be very effective in the treatment of sCRS, reserving the role of corticosteroids in patients who don't respond to tocilizumab. CAR T-cell therapy was used as a bridge to allo- SCT, 44% of patients could undergo allo-SCT.
- Maude et.al, from the Pennsylvania group subsequently reported on their expanded cohort of 30 patients who were treated with CTL019 cells containing the 4-1BB signaling domains. Thirty patients were treated, 25 patients were 5-22 years of age and 5 older patients. 26 had B-cell ALL in a first to fourth relapse, 3 had primary refractory B-cell ALL and 1 had T-cell ALL with aberrant CD19 expression. Twenty-seven patients had a complete remission, with 22 patients being MRD negative. 15 patients received no further therapy. CTL019 cells persisted for a longer duration upto 11 months, with a higher proliferation rate. The peak proportion of CTL019 modified T-cells were a median of 39%. The probability of relapse free B-cell aplasia at 6 months was 73%. CRS was seen in all the patients, 22 of whom had mild-moderate CRS and the rest had severe CRS. (Level of Evidence- 3B)

- Lee DW et al from the National Cancer Institute, Bethesda conducted a phase 1 dose escalation trial in relapsed ALL and lymphoma in children and young adults (1-30 years). 21 patients including 8 who had previously undergone allo-SCT were included. The maximum tolerated dose was defined as $1x10^6$/kg in entire cohort. The treatment was well tolerated and all of the toxicities were reversible. In the intention-to-treat analysis of ALL patients, 70% (14/20) had a complete response and 60% with a MRD negative remission (12/20). Overall survival was 51.6 % at 9.7 months. Ten patients underwent allo-SCT. Relapses were attributed to failure of CAR T-cell expansion, immunological mechanisms and loss of CD19 expression. This study correlated the risk of CRS to the tumor burden, response to Tocilizumab and the role of cytokines and CRP as predictive markers. (Level of Evidence- 3B)

Lympho-depleting Strategies:

Considerable differences are present between lympho-depleting strategies prior to CAR T-cell re-infusion, to prepare the host for the T-cell dose and allowing unrestricted growth in-vivo.

- The MSKCC groups use Cyclophosphamide as a single agent, and is studying the best approach in a forthcoming trial by introducing a conditional use of the drug allowing comparison. (Level of Evidence-3B)
- The NCI group has found it favorable to use Fludarabine and Cyclophosphamide combination as

an effective lymphodepletion strategy. Grade 3- 4 neutropenia is to be expected as a result of this strategy, and may last 8 days or more. Other groups may be moving to this strategy. Cyclophosphamide alone would be expected to contribute to less toxicity, though effect of CAR expansion in-vivo is unassessed.

Post-infusion Dynamics:

Post infusion, the CAR T-cells rapidly expand. Depending on the co-stimulatory domain used, peak level may be reached by 30-45 days, but levels of CD28 CARs rapidly decline thereafter, while the 4-1BB levels remained high for well beyond 90 days and could even be demonstrated at 143 days and beyond post infusion in murine models, and B-cell aplasia could be demonstrated in treated mice even on Day 209.

In humans too, similar effects have been seen with longevity of CAR T-cells in vivo and B-cell aplasia have correlated with type of co-stimulatory domain and both tend to be longer with the 4-1BB CAR. (Level of Evidence- 3B)

Current Trials:

Gains made in B-ALL are now being consolidated with Phase III trials being rolled out, while other malignancies are being explored for targeting. However, considerable heterogeneity exists in the target antigen, and the various components of the CAR construct, the vector used, and the processes and protocols used to manufacture and assess them. Also, innovators in the field having established "Proof of Principle" are rapidly tying up with Industry for their individual products and processes, which are then

being scaled up to large scale GMP manufacturing facilities to roll out Phase III trials. This is already pushing up costs dramatically, way beyond what an allo-SCT costs in our center. There is a real possibility that by the time CAR therapy becomes available in India, it would be even beyond the reach of those who can afford SCT.

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Immunotherapy in Non Hodgkin Lymphoma

Swaratika Majumdar, Hasmukh Jain

Introduction:

Non-Hodgkin lymphoma includes a wide and diverse group of lymphomas. Immunotherapy has made an impact in some of the subtypes and the data is mainly available for (1) diffuse large B cell lymphomas (DLBCL), (2) ALK positive large cell lymphomas (ALCL) and (3) low grade lymphomas including follicular lymphoma (FL), marginal zone lymphoma (MZL) and mantle cell lymphoma (MCL). CLL has already been dealt with previously.

Monoclonal antibodies:

Anti CD 20 antibodies have been used for long in *DLBCL, and Low Grade Lymphomas.* These strategies have mostly revolved around Rituximab, a chimeric monoclonal antibody against the CD20 B-cell antigen, often in combination with standard or newer chemotherapy agents. Ofatumumab and Obinutuzumab are other antibodies directed at CD 20 with clinical benefit. Monoclonal antibodies against other targets on B cell

lymphomas include Epratuzumab, a monoclonal antibody directed against CD 22 studied in Phase II setting in FL and DLBCL, and Dacetumumab, anti CD 40, which showed responses in refractory/recurrent NHL in a phase I trial as monotherapy. Some of these have been innovatively conjugated with intracellular cytotoxic agents for targeted delivery, such as Brentuximab vedotin (BV), an antibody-drug conjugate directed against CD 30 demonstrating impressive results in relapsed/refractory systemic ALCL irrespective of ALK status. It is also now used in relapsed refractory DLBCL including primary mediastinal B cell lymphoma (PMBCL) and post-transplant lymphoproliferative disorders (PTLD). However, monoclonal antibody mediated killing of cancer cells, though immunologically targeted, does not constitute Immunotherapy which implies harnessing of the innate and adoptive immune system of the host in some way to mediate cancer control, so these are not discussed here further in detail.

Bispecific T cell engager (BiTE):

A BiTE is a molecule consisting of a single polypeptide that possesses two specific antigen binding sites, one which engages a specific B-cell marker and another targeting a co-stimulatory on T cells allowing a T cell mediated killing of malignant B cell and sparing other cells. Blinatumomab, combining ani CD 19 and engaging CD3, showed an ORR of 69% was observed in all NHL subtypes and in 55% of patients with DLBCL. Interestingly, it has a very short half life (2 hours) and requires to be administered via continuous infusion for at least 4 weeks. Phase I/II studies

have adopted a step wise dose escalation strategy to target dose 112 µg/day to avoid adverse events including CRS, neurologic toxicity, and leukopenia/neutropenia. (Level of Evidence 2B)

Immune checkpoint inhibitors:

Tumour cells block immune responses and allow for self tolerance. Two of the T-cell inhibitory mechanisms involve the cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and programmed death 1 (PD-1) receptors. Monoclonal antibodies targeting CTLA-4 and PD-1 act by reducing the down-regulation of T-cell response against tumor cells.

In lymphoma, studies include targeting CTLA4 with ipilimumab and PD-1 with pidilizumab, pembrolizumab, and nivolumab.

Phase I trial of CTLA4-blockade with ipilimumab reported durable clinical responses of 31 and 19 months respectively in two patients with relapsed/refractory DLBCL and FL. Ipilimumab treatment causes high rates of diarrhea including grade 3/4 colitis

Pidilizumab following AHSCT in refractory DLBCL, PMBCL and transformed indolent B NHLs including FL showed impressive ORRs ranging from 51%-66% in phase I studies. This drug will be superseded by more specific PD 1 blockers, pembrolizumab, and nivolumab. Phase IB trials involving the former are underway while nivolumab showed objective response rates of 40% and 36% in FL and DLBCL respectively. Dose of nivolumab is 3mg/kg week 1 and week 4 followed by 2 weekly interval. It does not routinely require premedication. Reported toxicity includes

hepatitis, immune mediated pneumonitis, colitis, thyroiditis, and hypophysitis and proteinuria. (Level of Evidence 2B)

CAR (chimeric antigen receptor) T therapy:

(Level of Evidence 2B)

Mechanism and toxicity of CAR T Therapy has been described under section on CLL. Evidence for its use in NHL other than CLL will be presented here. CD19-specific CAR T cells have been studied in relapsed/refractory lymphomas as well as ongoing clinical trials for CD 30-specific CAR T cells.

National Cancer Institute (NCI) , USA first reported use of anti CD 19 CAR T in aggressive lymphomas namely DLBCL and transformed FLs, 8 out of 15 achieved CRs with durations ranging from 9-22 months.

Memorial Sloan Kettering Cancer Center (MSKCC) studied the use of CAR T cells as consolidative therapy following AHSCT. In this phase I study, six patients with poor-risk NHL underwent AHSCT with subsequent CAR T cell infusion. All patients obtained CR at first restaging following transplant and remained in remission at the reported median follow-up of 6 months.

Similar results were obtained from UPenn which included patients with FL, DLBCL and mantle cell lymphoma with relapsed/refractory disease and anticipated survival of less than 2 years. At 3 months, 18 patients had reported 67% ORRs and a 6-month PFS of 59%.

In a pivotal trial ZUMA 1, abstract in ASH 2017, presented the primary analysis of 111 patients with refractory aggressive B lymphomas treated with anti-CD19 chimeric antigen receptor (CAR) T cell, axi-cel . Patients received a target dose of 2×10^6 anti-CD19 CAR T cells/kg after low dose conditioning with cy/flu. ORR was 82% and at 8.7 months, 44% of patients were in response and 39% continued to be in CR. Grade e"3 CRS and neurologic events (NE) occurred in 13% and 28% of patients respectively.

Based on dramatic responses in Phase I/II studies in adult B NHLs, a CD19 directed retroviral vector CAR manufactured by Kite Pharma ® has recently gained USFDA approval for use in relapse/ refractory B-cell lymphomas in adults.

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Immunotherapy in Chronic Lymphocytic Leukemia

Swaratika Majumdar, Hasmukh Jain

Introduction:

Chronic lymphocytic leukemias (CLL) comprises of nearly 30% of adult leukemias in the Western world. Occurring at a median age of 70 years, it is reasonable to expect a cohort of patients with multiple comorbidities, poor performance status and poor tolerance to intensive chemotherapy.

Traditionally used regimens such as chlorambucil or fludaribine-cyclophosphamide have failed to demonstrate an improvement of OS. With advent of Rituximab, combination chemo-immunotherapy (FCR) showed remarkable OS benefit sparking of interest in immunotherapy centric management of CLL.

Rationale for the use immunotherapy:

1. CAR (chimeric antigen receptor) T therapy:

CD 19 forms an excellent target for CAR T cells as it is restricted to normal and malignant precursor B cells and spares the hematopoietic stem cell.

University of Pennsylvania infused CART 19 cells containing CD3æ activation domain and CD137 costimulatory domain (CTL019 cells) in relapsed/refractory CLL and demonstrated sustained remission upto 10 months after therapy. The preconditioning regimen for lymphocyte depletion was pentostatin 4 mg/m^2 and cyclophosphamide at a dose of 600 mg/m^2. The same group has since published results of 45 patients treated with CTL019 cells with reported ORR of 45%. They demonstrated functional CAR T cells at nearly 3 years after therapy but whether that translates to prolonged remission is yet to be determined.

So far clinical results with CD 19 directed CART cells are limited to small case series from Memorial Sloan Kettering and National Cancer Institute. MSK showed ORR in only 1/8 patients. NCI data evaluated 4 heavily pre-treated (3-7 prior lines of therapy) patients of CLL with CAR T 19 cells and all had controlled disease at 6-15+months of follow up. MSK data on the other hand was bleaker with only 1/8 demonstrating sustained partial response.

As CAR T cells are self-replicative, dose determining trial used a varying dose of 5 x 10^7 CTL019 cells to 5 x 10^8 CTL019 cells/kg. Other centres used a dose of 0.2-1.1 × 10^7 19-28z^+ T cells/kg.

Chief toxicities were typically delayed cytokine release syndromes (CRS) and tumour lysis syndrome (TLS). CRS typically occurred around day 22, driven by IL 6 and characterised by fever, myalgias and gastrointestinal symptoms. Potentially life threatening consequences like hypotension and hypoxia may occur as well. Efforts to

reduce CRS are underway specially developing a suicide switch modulating T cells activity. B cell aplasia and hypogammaglobulinemia have also been reported.

As an emerging alternative to allogenic transplant, CAR T therapy has the advantage of being devoid of graft versus host disease and not requiring long term immunosuppression. In elderly patients with relapsed refractory CLL, CAR T cells can be expected to yield sustained response rates. (Level of Evidence 2B)

2. Immune check point inhibitors:

Programmed cell death 1 (PD-1; CD279) and its ligands programmed death-ligand 1 (PD-L1; B7-H1; CD274) and PD-L2 (B7-DC; CD273) maintain a microenvironment that promotes tumour growth. In murine models, PDL1 check point blockade prevented development of CLL. Ibrutinib, BTK inhibitor, additionally inhibits th2 cellular responses and enhances antitumour immune responses of nivolumab (humanized IgG4 anti-PD-1 monoclonal antibody). Combination of ibrutinib and nivolumab is being tested in CLL. (Level of Evidence 3B)

3. Monoclonal antibodies:

Antibodies against TSA are most commonly immunotherapy in all B cell malignancies including CLL. Commonly engaged targets include: CD 19, CD 20 and CD 37 though only antibodies directed against CD 20 have clinical application at the moment. Antibodies engage with the TSA and mediate killing via antibody dependent or complement mediated cellular killing.

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Immunotherapy in Acute Myeloid Leukemia

Rajesh Bollam, Hasmukh Jain

Introduction:

Acute myeloid leukemia (AML) is a clonal, hemo¬poietic stem cell disorder which is characterized by the accumulation of immature myeloid precursors (blasts) in the bone marrow and peripheral blood, along with the suppression of normal hematopoiesis. It is the most common acute leukemia in adults, accounting for approximately 80 percent of adults. The standard regimens currently used to induce remission involve high dose induction chemotherapy with cytarabine and an anthracycline. Outcomes of AML with present therapy are dismal, 5 year over all survival is less than 40 % in age < 60 years, where as it is less than 10 percent in above 65 years Hence there is lot of scope for improvement in treatment strategies in AML. Immunotherapy is one the modality to improve treatment outcomes.

Immune Escape: During progression of tumor, certain tumor cells gain some characteristics, such as immortalization, resistance to apoptosis, independence of

growth signals. Loss of interaction between these tumor cells and host immunity plays an important role in this immune escape which helps in the tumor progression.

Treatment Modalities: Allogeneic HSCT, the most effective treatment of AML, is a form of Immunotherapy. However, many patients relapse after allogeneic HSCT and majority of elderly patients are not fir for HSCT. Hence alternative immunotherapeutic strategies are need of hour to treat patients who are not suitable for intensive treatment regimens as well as patients with relapsed and refractory aml.

Vaccination: Vaccination is one the most useful approach in preventing disease relapses who has achieved MRD (Minimal residual disease) negativity. Some of the examples of vaccinations which are commonly used in AML are peptide vaccinations, which are synthesized from tumor associated antigens. Few studies showing activitiy of Vaccination in AML are Oka et al and Kellihoz et al with WT1 peptide. Other approach of Vaccination is using autologous dendritic cells which are generated either from leukemia patients in CR loaded with Tumor antigens and differentiation of leukemiac blasts into Dendritic cells. Van Tendeloo showed exciting results with WT1 loaded dendritic cell vaccination. The results are these preliminary studies are encouraging but we need more studies in assessing the role of Clinical management of patients with AML in MRD negative status

Antibody drug conjugate : In this form of therapy, monoclonal antibody drug is conjugated with various toxins, which on entering cells, releases toxins in the acidic

medium of lysozymes and on reaching nucleus, Induces cell death through various mechanisms like DNA double strand break and cell cycle arrest. CD33 is used as target In AML. Gemtuzumab ozogamycin(G0) is one the example for antibody drug conjugate in which anti CD33 igG 4 Humanised antibody is attached to toxin called calicheamicin. USFDA approved its use in 2000.GO is studied in combination with Azacytidine and Histone de acytelator inhibitor vorinostat in phase 1 and phase 2 trials in relapse and refractory aml in elderly patient who are not fit for intensive chemotherapy. Response rates when combined with vorinostat and hypomethylating agents in around 30 to 40 %. The Major draw back of anti CD33 antibody is excess toxicity as CD33 is expressed in > 30 % healthy Bone marrow normal cells, to reduce these side effects SGN-CD 33A (vadastuximab talirine),has been used in trials. In this construct, a monoclonal anti-CD33 antibody is conjugated to a highly potent DNA-binding pyrrolobenzodiazepine dimer.CR Rate with this is around 50 % and it increases to 70 % when combined with hypomethylating agents. one of the reasons for increased response rate in combination with hypomethylating agents is that these agents increase CD33 expresion. SGN- CD33A when combined with HSCT –VOD is major concern and when it is combined with standard 3 +7 induction grade 4 myelosuppresion is a major problem.

Radiolabelled Antibodies: Radio immunotherapy uses Monoclonal antibodies which are conjugated with radioisotopes which directly emits radiation and this radiation kill malignant cells. b-Particle emitters have a

relatively long range (0.8-5.0 mm) and low linear energy transfer (approximately 0.2 kiloelectron volts/lm), which allows for the delivery of radiation to both the target cells and the surrounding cells, Hence used before HSCT. where as Alpha (a)-particle emitters have a much shorter range (50-80 lm) and higher linear energy transfer (approximately 100 kiloelectron volts/lm), which is more effective for targeting specific tumor cells without damaging the surrounding cells; therefore, this modality may be more useful for targeting residual disease or smaller tumor burdens. Eg MoAb M195. Currently, the most promising use of b-particle radioimmunotherapy may be the targeting of radiation preferentially to hematopoietic tissues in preparation for HSCT. Till date None of these agenst are approved by FDA for clinical use.

T cell-recruiting antibody constructs : These are a special class of molecules which are composed of the (scFv) single chain variable fragments of two antibodies of different specificity connected by a short peptide linker. Through simultaneous binding of a tumor-associated antigen and CD3µ in the T cell receptor complex, these molecules bring malignant cells and T cells in close proximity. The binding of CD3µ leads to T cell activation and expansion resulting in Granzyme B /perforin-mediated target cell lysis. The special feature of this strategy is that virtually any memory T cell can be recruited for target cell lysis irrespective of its specificity. Example : AMG 330, is a bispecific T cell engager (BiTE) construct targeting CD33. To reduce off target side effects, cd 123 is being tried and instead of BITE technology DART techonology is used (DART – dual affinity Re targetting)

CART Cells in AML: CARs are genetically engineered cell membrane-bound receptors that combine extracellular antibody binding and intracellular effector cell signaling, thereby enabling both MHC independent antigen binding and highly potent cytotoxic effector cell function. CART cells has become big success in all but same is not translated in AML because of non-restricted expression of AML associated antigens. CD 33 and CDC 123 are targets for CART therapy in AML. The toxicity associated with CD123 is less than cd 33 as it is less expressed on normal bone marrow cells.

Checkpoint inhibitors in AML : Many phase 1 and phase 2 trials are going in hematology in the use of check point inhibitors in AML, it has already emerged as one of options in solid malignancy and also in hodgkins lymphoma and various types of non hodgkins lymphoma, but still it is I infancy in AML. Various agents are being tried ias single agents and in combination with othera gents like Hypomethylating agents, cytarabine and HSCT in un favouable group. PD1, PDL-1 CTLA-4 are tried. None of them are approved yet for clinical use.

Hinderance to immunotherapy in AML : 1) lack of an AML-specific target antigen, 2) low mutational burden resulting in low endogenous immune responses 3) intrinsic resistancemechanisms of the leukemic blasts against immune responses

Future perspective: CombinatIon approaches are required for reversal of high relapse rate of AML. The focus lies on strategies for elimination of minimal residual disease in post remission therapy. Predictive biomarkers are needed

for the choice of immunotherapy. Clinical studies have to include immune parameters in order to identify novel biomarkers of immune efficacy.

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Immunotherapy for Malignant Melanoma

Sujeeth M, Jyoti Bajpai

Introduction:

Melanomas are tumors originating from melanocytes and tend to show early metastasis secondary to the loss of cellular adhesion in the primary tumor, resulting in high morbidity and mortality rates. The incidence of melanoma is increasing worldwide however, at present this is considered among the rare malignancies in India with the age adjusted incidence rate is 0.2 (per 100,000 population) and mortality rate is 0.1 (per 100,000 population) respectively.

Tumors can create antigens and neoantigens that are very different from those of normal tissues. Thus, elucidating these differences may be the key for developing customized immunological strategies with decreased side effects and increased immune responses. Cancer-specific active immunotherapy is a form of treatment that stimulates the immune system to recognize antigens on the surface of cancer cells.

Advances in immuno-oncology have improved our understanding of the interaction between the immune system, cancer cells, and the tumor microenvironment, and application of these discoveries has great significance for the treatment of melanoma.

Immunotherapy use in malignant melanoma is increasing worldwide based on rapid emergence of evidence of role of immune check point inhibitors (both as monotherapy and combination immunotherapy) in adjuvant and advanced settings. There are trials currently focusing on neoadjuvant and concurrent use of immunotherapy with radiation and chemotherapy. Immune check point inhibitors are recently got available in India and the experience in using these novel molecules are increasing; for these reasons till now there is no published literature from India on use of Immunotherapy in Melanoma.

Immunotherapy in Adjuvant setting:

Evolution of immunotherapy has changed the clinical practice in melanoma. Immune check point inhibitors (Antibody to PD-1 –nivolumab and antibody to CTLA-4-ipilimumab) have been used in the adjuvant setting.

Adjuvant ipilimumab at high doses (10mg/kg), every 3 weeks for 4 doses and then 12-weekly to complete 3 years was compared with placebo in a study. Only Stage III patients were enrolled in the study. It showed improved five-year relapse free survival (RFS) which was the primary endpoint of the study. It also showed improvement in secondary end points including overall survival (OS). 5 year RFS was 40.8% v/s 30.3% (EORTC 18071). But there were

significant immune related adverse events in ipilimumab arm. Grade 3 and above toxicities were seen in 54% patients and there were 5 treatment related deaths. Though in the study ipilimumab was not compared with interferon alpha the magnitude of benefit lead to its approval. Dose in this study is higher than that used in metastatic setting explaining the higher toxicities. (Level of evidence I).

Nivolumab (3 mg/kg), every 2 weekly for 1 year, has shown promise as adjuvant therapy for node positive resected advanced malignant melanoma. (Level of evidence I). In this study, nivolumab was compared to ipilimumab which showed significantly longer RFS (primary end point of the study) at 12 months (70% vs 60%) and a lower rate of grade 3 or 4 adverse events than adjuvant therapy with ipilimumab (14% vs 46%). (Checkmate 238). Longer follow up is required for overall survival data. Because of favorable toxicity profile nivolumab is the preferred choice for adjuvant immunotherapy in resected melanoma (Level of evidence 1)

High dose Interferon–alpha (20 MU/m^2 intravenous five days per week for four weeks, followed by 10 MU/m^2 subcutaneously three times per week for an additional 11 months) is FDA approved adjuvant therapy option in resected melanoma (stage IIB and stage III). When compared with placebo study showed 1 year survival benefit and 9% absolute survival benefit at 5 years. Adjuvant high-dose IFNa is associated with acute constitutional symptoms, chronic fatigue, myelosuppression, neurologic and psychologic effects,

which are experienced to some degree by the majority of patients. Due to the high toxicity potential is less preferred option in the era of other immune modulators with comparative favorable toxicity profile.

The results of chemotherapy alone have been dismal. However, Bio-chemotherapy (chemotherapy plus immunotherapy) has shown promising results. In SWOG S0008 trial, bio-chemotherapy (cisplatin, vinblastine, dacarbazine, IL-2, low dose IFN) for 9-week period was found to be superior to high dose IFN alfa-2b monotherapy for 1 year with respect to median RFS (4years vs 1.9years) and completion rate (80% vs 43%) respectively (Level of evidence IIB). Though no OS benefit was seen, toxicity profile was completely different in both arms. Also, toxicity lasted for 9 weeks period in bio-chemotherapy arm and for 1 year in interferon arm. This might be considered a useful option especially in resource constrained low and middle-income countries (LMICs).

Neoadjuvant Therapy

In a two-arm phase Ib feasibility trial, combination of ipilimumab (3mg/kg) and nivolumab (1mg/kg) in the neoadjuvant treatment setting for high risk stage III melanoma patients was found feasible and effective however, with higher grade 3/4 toxicity. The early phase data of increased efficacy at the expense of significantly increased toxicity in phase I trials merits more evidence from phase III trials before providing recommendations for routine clinical use.

Immunotherapy in Advanced Melanoma

In advanced melanoma, FDA approved immunotherapy options include monotherapy with Pembrolizumab (Antibody to PD-L1), Nivolumab, and Ipilimumab or dual therapy with Nivolumab with Ipilumumab.

Nivolumab or pembrolizumab monotherapy is preferable first line option considering favorable toxicity profile and its cost. However, one may consider dual therapy in good ECOG performance status patients demanding fast response.

Biomarkers like PD-L1 expression testing methods are currently being standardized and is not recommended routinely as PD-L1 inhibitors (Nivolumab, Pembroliumab) have shown to benefit irrespective of PD-L1 expression. Even response assessment with traditional RECIST is imperfect when immune checkpoint inhibitors are used.

Nivolumab: (level of evidence IA) showed improved response rate(RR), progression free survival (PFS) and OS compared with chemotherapy (dacarbazine) in previously untreated patients. One year OS of 73% v/s 42%. Nivolumab was shown to maintain baseline quality of life. (Checkmate 066). Similar benefit was shown with nivolumab in ipilimumab refractory patients. Nivolumab has been compared with ipilimumab in a study where there were 3 arms viz. ipilimumab plus nivolumab. Nivolumab and ipilimumab. This study was not powered to compare combination with nivolumab alone but it compared nivolumab containing arms to ipilimumab alone. Three year PFS were 39%, 32% and 10% respectively. Grade

3 and 4 adverse events were 59%, 21% and 28% respectively. (Checkmate 067).

Pembrolizumab: (level of evidence IA) showed improved RR and PFS compared to chemotherapy in ipilimumab refractory patients. In this study, there were 3 arms viz. pembrolizumab 2mg/kg every 3 weeks, pembrolizumab 10mg/kg every 3 weeks and chemotherapy arm in which both pembrolizumab arm showed improvement in primary end point of RFS at 24 months (16% vs 22% vs 0.6%). Another study also showed better OS and PFS than ipilimumab (monotherapy) in patients not treated with immune check point inhibitors before and may have received one line of therapy prior to enrolment. This study had 2 arms with pembrolizumzab (10mg/kg 2 weekly and 3 weekly) and 1 arm for ipilimumab (3mg/kg 3 weekly). Both pembrolizumab arms were equivalent in terms of results. Two-year OS was better in pembrolizumab arms 55% vs 50%. In a secondary analysis based upon patient-reported outcomes, quality of life and global health status were better maintained with pembrolizumab. (Keynote 002, Keynote 006)

Ipilimumab monotherapy or with nivolumab: (level of evidence IA) Ipilimumab has shown better OS in untreated patients when compared with chemotherapy and also in previously treated patients when compared with placebo. Ipilimumab/Nivolumab showed significantly improved RR and PFS when compared to Ipilimumab alone. (Checkmate 067, Checkmate 069).

Mucosal Melanomas

1) Mucosal melanoma is an aggressive malignancy with poor response to conventional therapies. Pooled analysis from clinical trials using immune check point inhibitors show that combination of nivolumab with ipilimumab seem to have higher efficacy than either agent alone in advanced mucosal melanoma. Median PFS and RR were found to be lesser than cutaneous melanoma (23 % vs 40%). The safety profile was however, similar between the two subtypes
2) Uveal melanoma: Immunotherapy has very limited clinical activity in metastatic UM, considered to be due to multiple factors including the tumor immune microenvironment, fewer mutation loadings, different mutational spectrum and different immunological aspects of tumors.

Melanoma with Brain Metastases:

The standard treatment for brain metastases in melanoma till recently was either neurosurgical removal if feasible or radiotherapy (whole brain RT or Steoreotactic radiosurgery). There were no effective systemic treatment options available. In 2017, early results of Immunotherapy became available.

In ABC phase II study, 53 asymptomatic patients with no prior therapy for their brain metastases were randomly assigned to either nivolumab plus ipilimumab or to nivolumab alone. RR to combination compared to monotherapy was 42% vs 20%. With a median follow-up of 16 months, responses appeared to be durable.

In Checkmate 204, 109 patients with asymptomatic brain metastases were treated with nivolumab plus ipilimumab. Based on an initial evaluation of the first 75 patients, the objective for intracranial metastases was 55%.

Thus, at present immunotherapy has revolutionised melanoma management and is indicated both in metastatic as well as adjuvant setting. However, cost being prohibitive to its routine use in resource limited settings.

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Immunotherapy in Breast Cancer

Rushabh Kothari, Seema Gulia

Introduction:

Breast cancer is the most commonly diagnosed cancer and major cause of cancer death among women in less developed countries, with 882,900 cases diagnosed in a year, accounting for 25% of cancer among females. In India, it is the most common malignancy in female with incidence of 145000 patients a year as per GLOBOCAN. Therapeutic treatments for breast cancer generally include surgery, chemotherapy, radiotherapy, endocrinotherapy and molecular targeted therapy. With the development of molecular biology, immunology and pharmacogenomics, immunotherapy has become a promising new field in breast cancer therapies. Studies have classified breast cancer into four subtypes: Luminal A (ER+/PR+/HER2-, grade 1 or grade 2), Luminal B (ER+/PR+/HER2+, or ER+/PR+/HER2- grade 3), HER2 overexpression(ER-/PR-/HER2+), and triple negative breast cancer (TNBC, ER-/PR-/HER2-).

The negative expression of ER, PR and HER2 in TNBC has unique biological characteristics and strong heterogeneity, no standard treatment but chemotherapy is suggested for the subtype. TNBC tumors are relatively poor prognostic as they have no actionable targets. Breast cancer is immunogenic, and multiple putative tumor-associated antigens, such as HER-2 and Mucin 1, are observed in the cancer. A subset of TNBC is amenable to immune modulation as per molecular signatures and hence there is a hope that check point inhibitors will have a role to play.

Metastatic setting:

Efforts to develop immune therapy for breast cancer patients have been going on for a long time but were futile until the modest success of PD-L1 or PD-1 checkpoint inhibitors in metastatic TNBC patients.

Keynote-012 trial, 32 patients with PD-L1–positive (e”1%) heavily pretreated TNBC received intravenous pembrolizumab (Antibody to PD-1) and demonstrated an overall response rate (ORR) of 18.5%. In this study PD-L1 level > 1 %s seen in 58.6% of the screened population. Grade 3 or higher toxicities were seen in 15.6% of the patients.

The phase Ib KEYNOTE-028 trial examined pembrolizumab in 25 patients with PD-L1–positive, ER positive, her2 negative tumors and reported ORR of 12%. Similarly, atezolizumab (Antibody to PD-L1) showed ORR of 19%(4/21 patients) in metastatic TNBC patients. All responding patients had PD-L1 expression of e”5%. Longer

follow up of patients treated with atelozizumab has shown durable responses. Survival at 1 year was better in responders (100% v/s 32%).

In the Javelin trial which included 168 metastatic breast patient refractory to or progressing after standard-of-care therapy unselected by cancer subtype and PD-L1 status. They received avelumab intravenously 10 mg/kg every 2 weeks. The confirmed objective response rate (ORR) was 3% overall (one complete response and four partial responses) and 5.2% in patients with TNBC. A trend toward a higher ORR was seen in patients with PD-L1+ versus PD-L1- tumor-associated immune cells in the overall population (16.7% vs. 1.6%) and in the TNBC subgroup (22.2% vs. 2.6%). Grade 3 or higher toxicity was seen in 13.7% patients.

The most promising results were seen with a combination of nabpaclitaxel and atezolizumab, where 32 metastatic TNBC treated with less than 4 lines were enrolled. Of these 24 were evaluable for response. Confirmed ORR was 42% and investigator assessed unconfirmed ORR was 71%. Some interesting observations in the trial were that responses were seen in patient not having PD-L1 expression. In responders, durable responses were seen. Also notable was higher Tumor infiltrating lymphocytes (TILs) in responders.

Neoadjuvant setting:

The phase II I-SPY 2 trial, 68 Her2- early or locally advanced breast cancer patients who merits neoadjuvant chemotherapy were enrolled. Mammaprint low and ER+

patients were excluded. Study showed that adding pembrolizumab in combination with standard chemotherapy (Paclitaxel followed by doxorubicin and cyclophosphamide) as a neoadjuvant treatment for locally advanced breast cancer, increased pathologic complete response (pCR) rate nearly threefold for TNBC (60% v/s 20%) and for ER+/Her2– (34% v/s 13%). Grade 3 or higher toxicities were seen in 5 patients.

Other immunotherapy options include cytotoxic T-lymphocyte– associated protein 4 (CTLA-4) blockade (minimal activity), combinations of PD-1 and CTLA-4 mAb (under study), Chimeric antigen receptor (CAR)–engineered T cells as adoptive therapy for breast cancer (under development) and therapeutic vaccination (negative randomized trial as adjuvant in advanced setting.

Trial	**Checkpoint inhibitor**	**Population**	**End point**	**PD-L1 status**
I-SPY2	Pembrolizumab (NACT)	LABC	Path CR TNBC (60% v/s 20%) ER+ (34% v/s 13%)	Not known
Keynote 12	Pembrolizumab	TNBC	ORR 18.5%	≥ 1%
Javelin	Avelumab	Unselected	ORR 3%	Unselected
Keynote 28	Pembrolizumab	ER+ Her2 neg	ORR 12%	≥ 1%
G027831	Atezolizumab	TNBC	ORR 19%	≥ 5%
GP28328	Atezolizumab and nabpaclitaxel	TNBC	ORR 42%	Unselected

Current immunotherapy is still in its infancy for breast cancer. As mentioned above, it cannot be denied that a portion of breast cancer patients can benefit from these immunotherapy strategies.

Preventive vaccines, Adoptive T cell therapy, CTLA4 checkpoint inhibitors are in the early stage of development and no recommendations can be made for same. Therapeutic vaccines have failed in randomized studies and aren't recommended.

Hence, As of now immunotherapy is best considered investigational (with no FDA approval) and may be considered after failure of standard line therapies in metastatic TNBC patients after discussion in multidisciplinary tumor board and communication with patient and family.

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Immunotherapy in Lung Cancer

Nikhil Pande, Kumar Prabhash

Introduction:

Lung cancer is the most commonly diagnosed cancer as well as the leading cause of cancer-related deaths worldwide. Particularly, lung cancer has an estimated incidence of 1.6 million new cases every year. Lung cancer patients are estimated to be around 70000 per year. Lung cancer is categorized into two major subtypes depending on their histological feature: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC and SCLC constitute 85% and 15% of total lung cancer cases respectively.

Essentially, surgery is the first-choice treatment, but most clinically diagnosed cases are inoperable. While chemotherapy and/or radiotherapy are the next considered options for such cases, these treatment modalities have limited benefit. For metastatic patient except EGFR mutated or ALK rearranged subset the prognosis is poor with median survival less than a year. Thus, new effective strategies with favorable toxicity profile are urgently needed.

Lung cancer management has undergone a major revolution over last 5 years with immunotherapy playing a lead role. Immunotherapy has made inroads from metastatic second line to first line and is now knocking doors to enter in management of locally advanced cancers which are treated with curative intent.

Seeing the available data it seems immunotherapy, in near future, will play a major role in management of lung cancer both in metastatic and locally advanced setting. Role of immunotherapy with radiation, combination of two immunotherapeutic agent or combination of chemo-immunotherapy are areas of active research.

Non-small cell lung cancer (Squamous and non-squamous histology)

Immunotherapy agents viz. pembrolizumab (antibody to PD-1) and nivolumab (antibody to PD-1) are approved in metastatic non-squamous NSCLC in both first and second line settings in driver mutation negative patients.

Pembrolizumab is approved in first line when PD-L1 expression is >50% in both squamous and non-squamous histology. (Level of evidence I). In Keynote-024 a phase III study, pembrolizumab monotherapy (200 mg IV 3 weekly) was compared with standard platinum doublet chemotherapy in 305 advanced NSCLC patients having PD-L1 positivity>50% who were EGFR and ALK negative. Progression free survival (PFS) was prolonged with pembrolizumab (10.3 vs 6 months, HR 0.50) which was the primary end point. Overall survival (OS) was prolonged with Hazard ratio(HR) 0.60. Overall response rate (ORR)

for pembrolizumab was 45% vs 28% in platinum doublet. In around 1650 patients screened PD-L1 level of > 50% was found in about 30% of the patients. Also grade 3-5 toxicities were less in pembrolizumab arm (27% v/s 53%).

Pembrolizumab is also approved in first line with chemotherapy (pembrolizumab/pemetrexed/carboplatin) in NSCLC. (Positive PD-L1 testing was not required) in non-squamous NSCLC. (Level of evidence II). In Keynote-021 a phase II trial, pembrolizumab (200mg 3 weekly) combined with pemetrexed + carboplatin was compared with same chemotherapy alone in 123 PD-L1 unselected advanced NSCLC patients in first line. ORR, the primary end point of the study, was 55% vs 29% favouring pembrolizumab combination. PFS was significantly prolonged at 13 vs 6 months. Also adverse event grade 3-5 were more with pembrolizumab combination (39% v/s 26%).

Pembrolizumab is also approved in second line both squamous and non-squamous NSCLC with PD-L1 expression >1%. (Level of evidence I). In Keynote -010 a randomized phase II/III trial assessed pembrolizumab in previously treated advanced NSCLC at 2mg/kg, 10mg/kg vs Docetaxel at 75mg/m2 every 3 weeks. The median OS was 10.4 months for lower dose, 12.7 months for higher dose and 8.5 months for Docetaxel. Based on this trial Pembrolizumab got a category I recommendation at 10mg/kg dose. Benefit was more obvious in group with PD-L1 level of > 50%. Even grade 3-5 toxicities were lower in pembrolizumab group(13% and 16% v/s 35%).

Nivolumab is approved in second line setting progressed on first line therapy in non-squamous NSCLC (Level of

evidence I). In CHECKMATE-057 phase III trial, 582 patients with first line platinum failure (or EGFR and ALK positive patients post first line TKI) were treated with nivolumab (3mg/kg every 2 weeks) or docetaxel (75mg/m2, 3 weekly). OS was prolonged with nivolumab 12.2 vs 9.4 months and 2-year OS was 29% vs 16%. The ORR for nivolumab arm was 19% vs 12% in docetaxel arm. Survival benefit was seen more in smokers. Even OS was improved only in patients expressing some PD-L1(>1%). In PD-L1 negative tumors there was no survival benefit. Grade 3 or higher toxicities were more with docetaxel.(10% vs 50%).

Nivolumab is also approved in second line setting progressed on first line platinum in squamous NSCLC (Level of evidence I). In the phase III CheckMate 017 trial, 272 patients with advanced squamous NSCLC who experienced disease progression during or after initial therapy with platinum-based doublet chemotherapy were randomly assigned to treatment with nivolumab (3mg/kg intravenously every two weeks), or docetaxel (75 mg/m^2 intravenously every three weeks). Overall survival (OS), the primary endpoint of the trial, was prolonged with nivolumab (median OS, 9.2 versus 6.0 months). The two-year OS rates with nivolumab versus docetaxel were 23 versus 8 percent. Surprisingly PD-L1 expression has no bearing on survival result in this subset of patient. Nivolumab failed to show benefit in first line setting when patients were selected with PD-L1 expression level of >5%.

Also, atezolizumab (antibody to PD-L1) is approved in metastatic squamous or non-squamous NSCLC progressed on first line therapy. (Level of evidence I). In OAK a phase

III trial enrolling 1225 PD-L1 unselected advanced NSCLC post one or more-line failure atezolizumab was compared with Docetaxel. OS was prolonged regardless of histology at 15.6 vs 11.2 months. 16% of patients had PD-L1 expression >50% and in them the median OS was 20.5 months vs 8.9 months with ORR of 31 vs 11%.

Small Cell Lung Cancer:

Immunotherapy is recommended as second line therapy for patients who have relapsed 6 months or less after primary therapy in small cell lung cancer. Both nivolumab alone or in combination with ipilimumab (anti CTLA-4 agent) are approved for this indication (Level of evidence II). In CHECKMATE-032 a phase II trial, 216 patients were treated with nivolumab or nivolumab + ipilimumab . ORR was achieved in 10% of patients on Nivolumab only vs 21% in combination. Toxicities were more with combination including 3 treatment related deaths.

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Immunotherapy in Gastrointestinal Malignancies

Vikas Ostwal, Nikhil Pande

Introduction:

Immunotherapy is now considered the fifth pillar of anticancer therapy beyond surgery, chemotherapy, radiotherapy and targeted therapy. The success of novel drugs targeting PD-1 and CTLA-4 which are immune checkpoint inhibitors represent a paradigm shift in cancer therapy. Targeting PD-1 affects lymphocytes rather than cancer cells and hence has anti-tumor activity against a myriad range of tumors by the activity of cytotoxic T lymphocytes (CTL).

CTLA-4 is a co-inhibitory molecule expressed on activated T cells and T regulatory (Treg) cells and interaction of CTLA-4 receptor with its ligand inhibit CD-28 mediated T cell stimulatory signals. PD-1 is another co-inhibitory receptor expressed on surface of activated T cells, T regs and monocytes interacting with its ligands, PD-L1 and PD-L2 to inhibit T cell activation. This eventually leads to down regulation of cellular and humoral immune responses. With antibody mediated blockage of PD-1 or PD-L1 we

achieve inhibition of this checkpoint ultimately resulting in T cell activation and antitumor activity. The FDA approval of Pembrolizumab in all advanced/metastatic mismatch-repair deficient or microsatellite instability-high (MSI-H) tumors irrespective of site is an unprecedented step from which a new 'tumor agnostic' biomarker has emerged.

Gastrointestinal (GI) cancers which includes esophagus and gastroesophageal junction (GEJ), gastric cancers, pancreatic cancers, cancers of liver (HCC) and biliary tract represent a group of heterogeneous disease with a huge disease burden worldwide. The burden of this disease cohort can be gauged from the fact that 25% of all GI cancers will present with upfront metastatic disease and 25-50% of patients will develop metastasis during the course of their treatment.

Colorectal cancers:

In colorectal cancers Pembrolizumab has been approved in MSI-H metastatic refractory cases (FDA approved in third line). However, it has not shown benefit in non MSI-H patients which constitute 85% of all metastatic CRC. In the study Pembrolizumab was administered intravenously at a dose of 10 mg per kilogram of body weight every 14 days in patients with mismatch repair–deficient colorectal cancers, patients with mismatch repair–proficient colorectal cancers The immune-related objective response rate (ORR) and immune-related progression-free survival (PFS) rate were 40% (4 of 10 patients) and 78% (7 of 9 patients), respectively, for mismatch repair–deficient colorectal cancers and 0% (0 of 18 patients) and 11% (2 of

18 patients) for mismatch repair–proficient colorectal cancers. (Level of Evidence II)

Nivolumab plus Ipilimumab phase II interim results in metastatic MSI-H CRC has also shown better ORR and overall survival (OS) versus both drugs individually in third line. The only study for non MSI-H patients showing clinical benefit was a phase Ib study of MEK inhibitor Cobimetinib with Atezolizumab showed ORR of 40% in third line metastatic MSI-S CRC.

Gastro esophageal and stomach cancers

In gastric and GE junction tumors, Pembrolizumab in PD-L1 positive recurrent or metastatic patients has been studied. 13% patients were having grade 3 or 4 AE (FDA approved in third line). In this study 39 patients were enrolled. 36 were evaluable for response. Eight (22%) patients were judged to have had an overall response at central review; all responses were partial. (Level of evidence II)

Nivolumab in a phase III study has also shown OS benefit with low AE in gastric and GEJ tumors in third line patients. In the study eligible patients (aged e”20 years with unresectable advanced or recurrent gastric or gastro-esophageal junction cancer refractory to, or intolerant of, standard therapy [including two or more previous chemotherapy regimens], with an Eastern Cooperative Oncology Group [ECOG] performance status of 0-1, and naive to anti-PD-1 therapy or other therapeutic antibodies and pharmacotherapies for the regulation of T cells) were recruited. Patients were randomly assigned (2:1) using an

interactive web response system to receive 3 mg/kg Nivolumab or placebo intravenously every 2 weeks, stratified by country, ECOG performance status, and number of organs with metastases. Median overall survival was 5.26 months, vs 4.14 months favoring Nivolumab. 12-month overall survival rates were also better with Nivolumab 26.2% vs 10.9%. Grade 3 or more toxicities with Nivolumab was 10% vs 7% compared to placebo.

Hepatocellular cancers

In HCC, Nivolumab has shown good response rates and OS benefit in second line in advanced HCC patients (FDA approved). Nivolumab versus Sorafenib as first line trial is ongoing and results awaited. (Level of evidence II)

Durvalumab has shown acceptable AE and antitumor activity and OS in second line HCC in phase I/II study. CTLA-4 agent Tremelimumab in patients with HCC due to Hep C has shown median OS, which was similar to Sorafenib making it the only tumor where CTLA-4 agents have shown, benefit as sole agents

Pancreatic cancers

In pancreatic cancers immunotherapy has only met with limited success and more robust data is awaited. Pembrolizumab has been FDA approved in MMR deficient pancreatic cancers in third line.

Phase Ib/II study of chemoradiation (CRT) alone versus CRT plus Pembrolizumab in resectable and borderline resectable pancreatic cancers (BRPC) has shown it to be safe. Patients with resectable or BRPC were randomized

to the investigational treatment to receive Pembrolizumab 200mg IV every 3 weeks on days 1, 22, and 43 during concurrent CRT with Capecitabine (825 mg/m2 orally twice daily, Monday-Friday, on days of radiation only) and radiation (50.4 Gy in 28 fractions over 28 days) to receive only concurrent CRT with Capecitabine. Restaging CT scan or MRI is performed at 4-6 weeks after completion of neoadjuvant treatment, and patients with resectable disease will undergo surgical resection. Post-neoadjuvant therapy, 6 patients had unresectable disease (3 on each arm), and 14 patients underwent surgery (10 in investigational arm vs 4).

Immunotherapy also includes the often-ignored vaccine therapy and Adoptive cells transfer which has however till now met with limited success. It is often very cumbersome with very high costs mostly and is still in very nascent stage with only anecdotal case reports and phase I/II data. But it may improve in times to come to provide formidable personalized weaponry against GI tumors.

Thus, Pembrolizumab is considered as a standard treatment option in MSI high metastatic colorectal cancer after failure of first 2 lines of therapy, PD-1 expressing metastatic gastroesophageal adenocarcinoma after failure of two lines of therapy. Nivolumab can be considered in Sorafenib failure Hepatocellular carcinoma. In all other tumors, currently, immunotherapy may be considered as experimental.

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Immunotherapy in Gynecological Malignancies

Shubhdeep Bose, Jaya Ghosh

Introduction:

Ovarian and cervical cancers are the most common gynecological cancers affecting women worldwide and in India. Cervical cancer is on a declining trend, but remains the second most common cancer in women after breast cancer. Every year in India, 122,844 women are diagnosed with cervical cancer and 67,477 die from this disease. The treatment of gynecologic cancers represents a therapeutic challenge, and there is an unmet clinical need for new therapies especially in platinum resistant epithelial ovarian carcinoma, metastatic cervical and metastatic endometrial carcinoma.

Though there is no robust data supporting immunotherapy in gynecological cancers, promising early data reported especially with immune checkpoint inhibitors make it likely that these agents will eventually become part of the treatment arsenal for gynecologic cancers.

Carcinoma Ovary:

Exploitable strategies include therapies to enhance tumor antigen recognition (tumor vaccines, innate immune activators) and therapies to enhance T cell activation (cytokines and immune checkpoint blockade).

In a phase II study of the anti–PD-1 antibody nivolumab, in 20 evaluable patients with platinum-resistant ovarian cancer were treated in 2 cohorts (with 1 or 3 mg/kg nivolumab every 2 weeks until progression or up to 48 weeks). Best overall response was the primary endpoint. Grade 3 or 4 adverse events occurred in 8 patients (20%) and two experienced severe adverse events (grade 3 disorientation, gait disorder, fever in 1 patient and grade 3 fever, deep venous thrombosis in the other). The best overall response rate(ORR) was 15%. Four patients experienced prolonged disease control (2 patients in each dose cohort) with 2 patients in the 3 mg/kg cohort experiencing a durable complete response (CR). While response rates were similar to what has been seen with chemotherapy in platinum resistant disease, the durable responses are atypical in this disease and a cause for enthusiasm particularly in a very heavily pre-treated population. PD-L1 expression did not significantly correlate with objective response. Fourteen of 16 patients with PD-L1 high expression did not show a response while 1 of 4 patients with low expression was a responder. (LEVEL OF EVIDENCE V)

Similar activity was reported for the PD-L1–blocking antibodies avelumab and pembrolizumab, with response rates ranging from 17% and 12%. The ongoing phase III

trial Javelin 200 which compares avelumuab, liposomal doxorubicin or a combination of both in platinum resistant or refractory ovarian cancer will answer an important question of the role of immunotherapy in this scenario.

Carcinoma Endometrium:

Endometrial cancer is molecularly divided in to 4 types viz. POLE, hypermutated copy number high and copy number low subtype. POLE and hypermutataed types have 15 to 7 fold more neoepitopes than the other two types. Even PD-L1 expression is high in hypermutated type. Based on this rationale checkpoint inhibitors hold promise in hypermutated(microsatellite instable) patient. In a study of pembrolizumab in non colorectal cancers, which included endometrial cancer patients, there was a 70% ORR. (LEVEL OF EVIDENCE II). In this study which evaluated efficacy of PD-1 blockade in patients with advanced MMR-deficient cancers across 12 different tumor types. Objective radiographic responses were observed in 53% of patients and complete responses were achieved in 21% of patients. Responses were durable with median progression-free and overall survival still not reached. Functional analysis in a responding patient demonstrated rapid in vivo expansion of neoantigen-specific T cell clones that were reactive to mutant neopeptides found in the tumor. These data support the hypothesis that the large proportion of mutant neoantigens in MMR-deficient cancers make them sensitive to immune checkpoint blockade, regardless of the cancers' tissue of origin.

Endometrial subset of Keynote 028 study enrolled female patients with locally advanced or metastatic PD-L1–

positive endometrial cancer who had experienced progression after standard therapy. Patients received pembrolizumab 10 mg/kg every 2 weeks for up to 24 months or until progression or unacceptable toxicity. Primary efficacy end point was ORR. Of 75 patients screened, 36 (48.0%) had PD-L1–positive tumors, and 24 (32.0%) were enrolled. Three patients (13.0%) achieved confirmed partial response. Three additional patients (13.0%) achieved stable disease, with a median duration of 24.6 weeks. Grade 3 treatment-related AEs were reported in four patients.

Carcinoma Cervix

Several phase I/II studies with checkpoint inhibitors are ongoing in recurrent, metastatic platinum refractory setting. No results are yet available.

Recently, a study investigating a therapeutic vaccine employing E7 and L2 has demonstrated strong L2-specific IgG humoral responses while still providing modest T cell mediated immunity. This provides excellent prospect for the future of DNA vaccineresearch capable of generating both preventive and therapeutic effects against HPV infections.

Pembrolizumab was found to be useful for unresectable drug resistant/refrectory gestational trophoblastic neoplasia (GTN) in a case series by Ghorani et al (Charing Cross Hospital, UK). The efficacy and acceptable toxicity of Pembrolizumab makes it an alternative in this otherwise fatal cohort.

Summary and Recommendations

In relapsed carcinoma ovary immune checkpoint inhibitors are presently under investigation and not approved outside of clinical trial. (level of evidence V)

In recurrent/metastatic carcinoma endometrium pembrolizumab can be considered for MMR deficient patients who have progressed on prior therapy and do not have a satisfactory alternative treatment. (level of evidence II)

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Immunotherapy in Head and Neck Cancer

Vijay Patil, Amit Joshi

Introduction:

Head and neck cancers are amongst the commonest malignancies in our country especially in males. It amounts to almost one third of all malignancies. Limited treatment options in relapsed/metastatic setting and poor prognosis of these patients have led to search of novel approaches. Immunotherapy, and specifically checkpoint inhibitors have been one of them.

Now immunotherapy agents are approved in metastatic, recurrent or platinum refractory Head and Neck tumours. Immunotherapy agents approved are antibody to PD-1 viz. nivolumab (Level of evidence I) and pembrolizumab (Level of evidence II).

Nivolumab:

In Checkmate-141 phase III trial, 361 patients with recurrent squamous-cell carcinoma of the head and neck whose disease had progressed within 6 months after

platinum-based chemotherapy to receive nivolumab (at a dose of 3 mg per kilogram of body weight) every 2 weeks or standard, single-agent systemic therapy (methotrexate, docetaxel, or cetuximab). The primary end point was overall survival. Median follow up of 5.1 months overall survival (OS) was significantly longer in nivolumab arm with one-year survival at 36% vs 16.6. %. The overall response rate (ORR) was also increased with nivolumab (13.3 vs 5.8%). The benefit was maximum when PD-L1 expression e" 1 % and OS benefit was not significant in those with PD-L1 <1 %. Grade 3 or higher toxicities were lower with nivolumab. (13% v/s 35%).

Pembrolizumab:

In Keynote -055 phase II study, 171 patients who had failed prior platinum and cetuximab therapy were treated with pembrolizumab (200 mg every 3 weeks). ORR was 16% with a median duration of response of 8 months and 75% of responses were ongoing at time of analysis .Median progression free survival (PFS) was 2.1 months and OS of 8 months.

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Immunotherapy in Renal Cell Cancer

Kumar Prabhash, Amit Joshi

Introduction:

Renal cell cancer (RCC) comprises a diverse group of solid tumors originating from renal parenchymaRCC, with a globally rising incidence, is the seventh most common cancer in men and the ninth most common in women and constitutes approximately 2–3% of adult malignancies. In India, the estimated incidence of RCC among males is about 2/100,000 population and among females is about 1/100,000 population. Almost, half of patients, at the time of presentation, have locally advanced or metastatic disease.

Several approaches of active and passive immunotherapy have been studied extensively in clinical trials of patients with RCC. Treatment for metastatic renal cell carcinoma was limited few years ago but now we have more than 5 FDA approved drugs for second line use in metastatic setting. Immunotherapy has established itself in this role.

Checkpoint Inhibitors:

Checkpoint inhibitors are being studied in adjuvant, frontline metastatic setting and along with radiation in renal cell carcinoma. In future they may play a role in these settings also.

Immunotherapy agent nivolumab (antibody to PD-1) is approved in renal cell cancer clear cell histology patients who have received at least one prior anti-angiogenic therapies (approved in second line)

In phase III Checkmate 025 trial, 821 patients with advanced clear-cell renal-cell carcinoma for which they had received previous treatment with one or two regimens of antiangiogenic therapy were randomly assigned to receive 3 mg of nivolumab per kilogram of body weight intravenously every 2 weeks or a 10-mg everolimus tablet orally once daily. The primary end point was overall survival(OS). The trial was stopped early after a planned interim analysis showed improvement in overall survival. OS was significantly increased in nivolumab arm, median 25 vs 19.6 months with hazard ratio (HR 0.73). The overall response rates(ORR) was greater with nivolumab 25 vs 5% with fewer grade 3/4 adverse events(AE) 19 vs 37%.

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Immunotherapy in Bone and Soft Tissue Sarcoma

Akhil Kapoor, Jyoti Bajpai

Introduction:

Sarcoma is a group of heterogeneous tumors comprising of more than 50 different tumors. Adult Sarcomas are considered rare malignancies with an incidence rate of less than 1%. Advanced and recurrent sarcoma has limited treatment options. Immunotherapy is being explored in this setting with limited success yet.

Different subtypes of soft tissue and bone sarcomas have been shown to express PD-1 ligand. Paoluzzi et al conducted a retrospective analysis of 28 patients of sarcoma who received at least 4 doses of nivolumab (Antibody to PD-1). Overall response rate (RR) was 50% of the evaluable patients with 3 partial responses (PR) (12.5%). Another study failed to demonstrate antitumor activity of nivolumab among metastatic leiomyosarcoma patients.

A multicenter phase II study by Twabi et al enrolling 86 advanced/ unresectable or metastatic sarcoma patients

who have received upto 3 lines of chemotherapy. Median follow-up was 17.8 months (IQR 12.3–19.3). Seven (18%) of 40 patients with soft-tissue sarcoma had an objective response, including four (40%) of ten patients with undifferentiated pleomorphic sarcoma, two (20%) of ten patients with liposarcoma, and one (10%) of ten patients with synovial sarcoma. No patients with leiomyosarcoma (n=10) had an objective response. Two (5%) of 40 patients with bone sarcoma had an objective response, including one (5%) of 22 patients with osteosarcoma and one (20%) of five patients with chondrosarcoma. None of the 13 patients with Ewing's sarcoma had an objective response (Level of evidence V).

NY-ESO-1 is expressed in about 60% of synovial sarcomas. A pilot study by Mackall et al evaluated NY-ESO-1c259T cells recognizing an NY-ESO-1 derived peptide complexed with HLA-A*02 and have reported promising efficacy and acceptable safety.ORR was 50% (1 CR; 5 PR). Time to response was 6 weeks (range 4-9) and median duration of response was 31 weeks.

A new class of vaccines that uses an integration-deficient lentiviral vector to selectively target CD209 on DCs via its envelope glycoprotein derived from Sindbis virus appears to induce strong T-cell responses in trials that target NY-ESO-1 in SS and MRCL. Phase I/II studies of this vaccine showed encouraging 1-year overall survival of 82%. Sarcomas being superficial, Talimogenelaherparepvec, an oncolytic virus approved by the US Food and Drug Administration for melanoma, may be well suited to soft tissue sarcomas for injection at bedside. (ClinicalTrials.gov identifier: NCT02453191). The addition of anti–PD-1 in the

metastatic/refractory setting may potentiate activity upon the sarcoma tumor microenvironment (ClinicalTrials.gov identifier: NCT03069378).

Thus, at present, no form of immunotherapy is approved for soft tissue sarcomas outside the purview of a clinical trial and can be considered only in metastatic/ refractory setting when other treatment options are exhausted after thorough discussion with family.

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Immunotherapy in Micro-Satellite Instable- High (MSI-H) Tumors

Nikhil Pande, Vanita Noronha

Introduction:

Microsatellite instable tumors are those who express more neoantigens than microsatellite stable tumors of the same site. Due to increase neoantigen expression they are amenable to immunomodulation. Pembrolizumab – FDA approved for microsatellite instability – high (MSI-H) or mismatch repair deficient (dMMR) tumors. This indication covers patients with solid tumors that have progressed following prior treatment and who have no satisfactory treatment options. This is unique approval, as indication is not based on site of tumor but biology of tumour.

Data supporting this comes from following 15 cancers: colorectal cancer, endometrial, biliary, gastric and gastro esophageal junction, pancreatic, small intestinal, breast, prostate, bladder, esophageal, sarcoma, thyroid, retroperitoneal adenocarcinoma, small cell lung and renal cancer.

Overview:

In a phase II study in NEJM, there were 3 cohorts: 11 patients with dMMR colorectal cancers(CRC), 21 patients with MMR proficient and 9 patients with other tumors with dMMR. Pembrolizumab was administered intravenously at a dose of 10 mg per kilogram of body weight every 14 days. Overall response rate (ORR) was seen in 40% patient with dMMR CRC, 11% for 2^{nd} cohort and 67% for other dMMR tumors. Median overall survival(OS) was not reached in both cohorts with dMMR at 36 weeks of follow up while it was 5-month in MMR proficient CRC. Whole-exome sequencing revealed a mean of 1782 somatic mutations per tumor in dMMR tumors, as compared with 73 in mismatch repair-proficient tumors, and high somatic mutation loads were associated with prolonged progression-free survival.

In another phase II study in Science, 86 patients with dMMR across 12 tumor types who had at least failed 1 prior therapy. ORR occurred in 53% and of which 21% had radiologic complete response (CR). Median progression free survival (PFS) and OS was not reached after median follow up of 12.5 months.

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Immunotherapy in Urothelial Cancers

Vanita Noronha, Amit Joshi

Introduction:

The American Cancer Society estimates 79030 new cases and 16870 deaths from urothelial carcinoma in 2017. In India, it is ninth common malignancy accounting for 3.9% of all cancer cases. Clinically, urothelial carcinoma can be divided into non-muscle-invasive bladder cancer (NMIBC), muscle-invasive bladder cancer (MIBC), and metastatic urothelial carcinoma (mUC).

In urothelial carcinoma management, either in metastatic or recurrent setting, the only treatment approved was platinum based chemotherapy and many patients were not eligible to take them due to poor general condition or renal derangement. Patients who had progressed after short platinum free interval had limited treatment options. Immunotherapy with checkpoint inhibitors have fulfilled these unmet needs.

Immunotherapy agents are approved for treatment of locally advanced or metastatic urothelial cell carcinomas in following situations

1. Not eligible for cisplatin containing chemotherapy in first line treatment
2. Those who have progressed during or after platinum based chemotherapy in second line
3. Progression within 12 months of neoadjuvant or adjuvant platinum containing chemotherapy

Immunotherapy agents approved are antibody against PD-1 like pembrolizumab, nivolumab and antibodies against PD-L1 like atezolizumab, durvalumab, avelumab. (Level of evidence I)

Antibody to PD-1:

In Keynote -045 phase III trial, 542 patients who had recurred or progressed on platinum containing regimen were randomly assigned to pembrolizumab at a dose of 200 mg every 3 weeks or investigator choice chemotherapy. Patients were enrolled regardless of PD-L1 levels. Overall Survival(OS) was significantly increased with pembrolizumab at 10.3 vs 7.4 months and response rates (RR) were also high at 21 vs 11%. Grade 3 or more adverse effect were also fewer in pembrolizumab arm (15% vs 49.4%).

In Keynoye-052 phase II study, 370 patients who were not eligible for a cisplatin-based regimen either due to comorbidities, age or were treated with pembrolizumab 200 mg every 3 weeks. At a median follow-up of 9.5 months the RR the primary endpoints was 29% with 7% complete response(CR). Thus, first-line pembrolizumab has antitumour activity and acceptable tolerability in cisplatin-

ineligible patients with urothelial cancer, most of whom were elderly, had poor prognostic factors, or had serious comorbidities.

In Checkmate-275 a phase II study, 270 patients were treated with nivolumab in second line setting. The RR was 19.6% and at seven month follow up the OS was 8.7 months.

Antibody to PD-L1:

In a phase II trial 310 patients were treated with atezolizumab in second line setting. RR was seen in 15% of patients with 84% response ongoing at 12 months of median follow up. RR in patients with >4% PD-L1 expression was 27%. Grade 3-4 treatment-related adverse events, of which fatigue was the most common (five patients [2%]), occurred in 50 (16%) of 310 treated patients. Grade 3-4 immune-mediated adverse events occurred in 15 (5%) of 310 treated patients, with pneumonitis, increased aspartate aminotransferase, increased alanine aminotransferase, rash, and dyspnoea being the most common. No treatment-related deaths occurred during the study.

In multicenter single arm phase II study 1200 mg intravenous atezolizumab every 21 days until progression was used in first line in 119 patients who were treatment naive with locally advanced or metastatic urothelial cancer who were cisplatin ineligible. RR was 23% with 9% in CR and the median OS for the entire cohort was 16 months.

Durvalumab was studies in a phase I/II study where 191 patients were treated in second line. RR was 18% with 7 CR . One year OS was 55%.

Avelumab was evaluated in a phase I expansion cohort of 44 patients who were followed for a median of 16.5 months. The RR was 18% with 3 patients with PR and 5 patients in CR. The median OS was 13.7 months.

A subcutaneous model of bladder cancer has shown PD-1 blockade combined with the streptavidin–granulocyte-macrophage-colony stimulating factor (GM-CSF) -modified MCSCs (single cells extracted from MB49 cells) vaccine could induce better antitumor immunity than the vaccine or PD-1 blockade alone can. The findings may provide an experimental basis for applying this type of combination therapy to the treatment of human bladder cancer in near future.

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