Title: Cancer Immunotherapy in Special Challenging Populations: Recommendations of the Advisory Committee of Spanish Melanoma Group (GEM)

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SUPPLEMENTARY APPENDIX

This appendix has been provided by the authors to give readers additional information about their work.

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SUPPLEMENTARY DATA: INCIDENCE AND PATHOPHYSIOLOGY

1. HIV-1 INFECTION

HIV-1 virus produces a rapid depletion of $CD4^+$ T cells, both due to a direct cytopathic effect on infected $CD4^+$T cells, as well as an immune mediated effect on uninfected bystander[1] CD4+ T cells, while CD8+ T cell counts increase due to an expansion of oligoclonal $CD8^+$T cells, leading to a low ratio of $CD4^+/CD8^+$ T cell counts. When patients are treated with antiretroviral drugs (ART), $CD4^+$T cell counts usually increase and are recovered, but in some cases, mainly if ART is initiated late in the course of the infection, $CD4^+$ T cell counts could remain low. Moreover, patients who recover $CD4^+$T cell counts, can also retain high $CD8^+$ T cell counts, leading to a low $CD4^+/CD8^+$ ratio with normal $CD4^+$ T cell counts.[2] The normal $CD4^+/CD8^+$ ratio in healthy persons is not fully defined, but usually it is above 1.5.[3] In persons infected by HIV-1 a low $CD4^+/CD8^+$ ratio correlates with a stage of immune disfunction and chronic inflammation with a higher risk of mortality.[4]

In the prospective study conducted by Uldrick et al, inclusion of patients with less than 200 $CD4^+$ T cells/mm^3 was allowed when they had a $CD4^+/CD8^+$ ratio above 0.4.[5]

2. VIRAL HEPATITIS

Around 30% and 95% of adults infected by HCV or HBV, respectively, will spontaneously clear the virus. Despite multiple HBV plasma markers, the most important are hepatitis B surface antigen (HBsAg), which is the marker of chronic hepatitis B infection, and the antibody against hepatitis core protein (anti-HBc), which is the marker of previous exposition to the virus. Anti-HBc antibody is positive in all HBsAg positive patients (chronic hepatitis B), but also in subjects with resolved infection (HBsAg negative with anti-HBc positive). Analysis of HBV DNA is mandatory in all HBsAg positive patients in order to assess the stage of the infection. Moreover, HBV DNA should also be carried out in HBsAg negative with anti-HBc positive cases since, although uncommon, 0.1%-2.4% of these patients have detectable HBV DNA , the so-called “occult HBV infection”, and they have an increased risk of HBV reactivation when they undergo immunosuppression **(Supplementary Table 1)**.[6]

3. TUBERCULOSIS INFECTION

Most TB cases are caused by Mycobacterium tuberculosis (MT). The bacillus is carried in airborne particles, called droplet nuclei, of 1– 5 microns in diameter and they are generated when persons who have pulmonary or laryngeal TB disease cough, sneeze, shout, or sing.[7] When the tubercle bacilli reach the alveoli of the lungs, they are ingested by alveolar macrophages that form a barrier shell, called granuloma. Although most of these bacilli are destroyed or inhibited, a small number of them can multiply intracellularly, and they are released when the macrophage cell dies. These bacilli may spread through the lymphatic channels or the blood stream to more distant tissues and organs (regional lymph nodes, apex of the lung, kidneys, brain, and bones).[7] In summary, there are three clinical situations: infection eliminated, latent TB infection (LTBI) and subclinical or active TB disease. People with LTBI cannot spread the infection to other persons. Within weeks after infection, the immune system is usually able to stop the multiplication of the bacilli, preventing further progression.[7] Conversely, persons with TB disease are usually infectious and may spread the bacteria to others. It is caused because the tubercle bacilli overcome the immune system and multiply.[7] Without treatment, approximately 10% of persons with a normal immune system infected by MT will develop TB disease (5% in the first or second year, and 5% later in life). Some people are at higher risk than others to develop TB disease, for example persons with non-treated HIV infection and people who are receiving immunosuppressive therapy.[7]

People with LTBI do not have any symptoms. When TB bacilli grow in the lungs, it causes symptoms, such as: cough that lasts 3 weeks or longer, pain in the chest and coughing up blood or sputum. Other symptoms of TB disease may include: weakness or fatigue, weight loss, no appetite, chills, fever and night sweats. In 15-20% of cases, TB affects other organs, so they have additional symptoms and complications, including, meningitis, ascites, Pott's disease (TB spondylitis), scrofula (lymphadenitis) and genitourinary alterations.[7]

Mantoux tuberculin skin test (TST) is performed by intradermal injection of tuberculin purified protein into the inner surface of the forearm. Usually, the injection should produce a pale elevation of the skin (a wheal) 6 to 10 mm in diameter. The skin test reaction should be read between 48 and 72 hours after administration. The reaction should be measured in millimeters of the induration, but not measuring the erythema. The diameter of the indurated area should be measured across the forearm (perpendicular to the long axis).

An induration of 15 or more millimeters is considered positive in everyone. An induration of 10 or more millimeters is considered positive in recent immigrants (< 5 years) from high-prevalence countries, residents and employees of high-risk congregate settings, injection drug users, bacteriology laboratory personnel and children less than 4 years old.

Gonzalez-Cao M, *et al. J Immunother Cancer* 2021; 9:e001664. doi: 10.1136/jitc-2020-001664

SUPPLEMENTARY TABLES

Table S1. Summary of the main studies and reviews

Author	Condition	n	year	Cancer Type	IO	DCR	IrAESs G3-4	Disease reactivation	Study type
Uldrick et al[5]	HIV	30	2019	Anal cancer, NHL, others	Pembrolizumab	17%	20%	0	Prosp
Gonzalez-Cao et al[8]	HIV	20	2020	NSCLC, melanoma, others	Durvalumab	50%	0%	0	Prosp
Gonzalez-Cao et al[9]	HIV	44	2018	Melanoma, NSLC, HCC, others	Nivolumab Pembrolizumab Ipilimumab	25%	18%	0	Review of case series and case reports
Cook et al[10]	HIV	73	2019	Melanoma, NSCLC, Kaposi, others	Nivolumab Pembrolizumab Ipilimumab	27-63%	8.6%	7%*	Review of case series and case reports
Zhang et al[11]	HBV	114	2019	HNC, nasopharyngeal, melanoma, others	Pembrolizumab Nivolumab Atezolizumab	NA	2%	5%**	Retrosp
Gane et al[12]	HBV	24	2019	No cancer	Nivolumab	NA	0	90% decline viral DNA	Prosp
Ziogas et al[13]	HBV	10	2020	Melanoma, NSLC, HCC, others	Pembrolizumab Nivolumab Ipilimumab Others	NA	NA	reports cases with HBV reactivation	Review of case series and case reports
Lee et al[14]	HBV	60	2020	HCC	Pembrolizumab Nivolumab		3% (ir Hepatitis)	0/54 with NUC therapy 1/6 without NUC	Case series

								therapy	
Sangro et al[15]	HCV	20	2013	HCC	Tremelimumab	76%	0	75% decline viral RNA	Prosp
El-Khoueiry et al[16]	HCV/HBV	50/51	2017	HCC	Nivolumab	66%/55%	8%/0%	transient reduction	Prosp
Davar et al[17]	HCV	2	2015	Melanoma	Pembrolizumab	50%	0	stable pVL	Case Report
Van Eeden et al[18]	TB	12	2019	NSCLC, melanoma, others	Nivolumab Pembrolizumab	NA	NA	reports cases with TB reactivation	Cases Serie
Fisher et al[19]	SOTR	57	2020	Melanoma, others	Nivolumab Pembrolizumab	25%-60%	NA	37% Graft rejected 14% deaths due to graft rejection	Review of case series and case reports
Manohar et al[20]	SOTR (Renal transplant)	44	2020	Melanoma, SQCC skin, Merckel, others	Nivolumab Pembrolizumab Ipilimumab Avelumab	46%	NA	41% Graft rejected 44% deaths due to graft rejection	Review of case series and case reports
dìzarny et al[21]	SOTR	83	2020	Melanoma, NSCLC, SQCC skin, others	Nivolumab Pembrolizumab Ipilimumab Avelumab	31%	18%	40% Graft rejected 18% deaths due to graft rejection	Review of case series and case reports
Burotto et al[22]	Pregnancy	1	2018	Melanoma	Ipilimumab plus Nivolumab (1st trimester)	PD	immune related Hepatitis	No pregnancy complications	Case report
Xu et al[23]	Pregnancy	1	2019	Melanoma	Nivolumab (1st trimester)	CR	immune related Tiroiditis	No pregnancy complications	Case report
Mehta et al[24]	Pregnancy	1	2017	Melanoma	Ipilimumab plus Nivolumab (1st trimester)	PD	No irAE	No pregnancy complications	Case report
Coureau et al[25]	AD	191	2020	Melanoma NSCLC	Nivolumab Pembrolizumab	12%-50%	30%	27% -50% flare up of AD	Review of case

				others	Ipilimumab				series and case reports
Abdel Wahab et al[26]	AD	123	2018	Melanoma NSCLC others	Nivolumab Pembrolizumab Ipilimumab plus nivolumab Atezolizumab Others	NA	25%	41% flare up of AD	Review of case series and case reports

*Footnotes:*Became detectable pVL **Without antiviral prophylaxis*
Abbreviations: AD: autoimmune diseases; NA: no data available; NHL: non Hodgkin lymphoma; HNC: head and neck carcinoma; NSCLC: non-small cell lung cancer; Prosp: Prospective; Retrosp: Retrospective; SOTR: solid organ transplant recipients; SQCC: squamous cell carcinoma; TB: tuberculosis;

Table S2. Simplified interpretation of the serological status for Hepatitis B.

	Anti-HBc	Anti-HBc IgM	HBsAg	Anti-HBs	HBV DNA	ALT
Acute infection	+	+	+	-	Detectable	Very increased
Chronic infection B	+	-	+	-	Detectable	Normal
Chronic hepatitis B	+	-	+	-	Detectable	Increased
Resolved hepatitis B	+	-	-	+/-	Undetectable	Normal
Occult hepatitis B	+	-	-	+/-	Detectable*	Normal
Vaccination	-	-	-	+	Undetectable	Normal
Not vaccinated neither exposed	-	-	-	-	Undetectable	Normal

* Usually low HBV DNA values (< 200 IU/mL).

Footnote: For the diagnosis of chronic HBV infection, several determinations in plasma or serum are used: hepatitis B surface antigen (HBs Ag), hepatitis B e antigen (HBe Ag), antibody against HBs Ag (anti-HBs), antibody against hepatitis core protein (anti- HBc) and the determination of HBV DNA by PCR. The presence of HBV DNA in serum or plasma is the gold standard for the diagnosis of the infection, as it denotes active infection and correlates with the infectivity of the patient. Nevertheless, for screening of a HBV infection, serological tests are initially indicated, and determination of viral DNA is reserved for doubtful cases. Chronic HBV infection is defined by the presence of HBs Ag in the blood for longer than six months. If negative, chronic HBV infection is typically ruled out. As Anti-HBs is a neutralizing antibody that confers protective immunity, it can be positive in previous vaccinated patients without infection and also in persons that have overcome the infection. The difference between both situations is that only persons that have been infected have detectable anti-HBc antibodies. It is very infrequent, but possible, that persons with detectable anti-HBs antibodies and HBs Ag positive are chronic carriers. The presence of HBe Ag indicates active viral replication and it does not discriminate between acute or chronic hepatitis. The presence of Anti-HBc indicates a prior exposure to the virus, but it is not a neutralizing

antibody and it does not discriminate between acute or chronic infection. In some cases, anti-HBc antibody Ig G can be the only positive marker of infection, as in the window period of acute infection, but also in some cases with chronic infection. This peculiar serological profile occurs in 1% in low-prevalence countries, but close to 20% of blood donors in countries with endemic infection. The presence of an isolated IgG anti-HBc may suggest an occult HBV infection. Occult HBV infection is currently defined as the absence of circulating HBsAg in individuals positive for HBV *DNA. Usually, if anti-HBc is negative, a chronic infection can be ruled out, but in immunocompromised chronically infected patients it can be negative, although viral DNA is positive. More than 20% of the occult carriers are negative for all serum markers of HBV infection and they are called "seronegatives".*

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