UNIVERSITÀ DEGLI STUDI DI MILANO

CONCORSO PUBBLICO, PER TITOLI ED ESAMI, A N. 1 POSTO DI CATEGORIA D – AREA TECNICA, TECNICO-SCIENTIFICA ED ELABORAZIONE DATI, CON RAPPORTO DI LAVORO SUBORDINATO A TEMPO INDETERMINATO PRESSO L'UNIVERSITÀ DEGLI STUDI MILANO - COSP - CENTRO PER L'ORIENTAMENTO ALLO STUDIO E ALLE PROFESSIONI – CODICE 22179

La Commissione giudicatrice del concorso, nominata con Determina Direttoriale n. 17389 del 21.11.2022, composta da:

Prof. Muzi Falconi Marco	Presidente
Dott.ssa Landoni Michela Veronica	Componente
Dott.ssa Pastori Valentina	Componente
Dott.ssa Iacopino Lucia	Segretaria

comunica i quesiti relativi alla prova orale:

GRUPPO DI QUESITI N. 1

1 In 10 minuti, spieghi ad una classe di liceo cos'è e come funziona il sistema CRISPR/Cas.

2 Descriva il progetto di ricerca di cui si è occupato/a nel corso della sua ultima esperienza in un laboratorio di ricerca.

3 Legga e traduca il brano in inglese presentato dalla commissione, tratto da DNA Repair vol 120:10349.

Brano in inglese: The immune system is a powerful network of cells and proteins that function to protect the body against illness and infection caused by pathogens such as virus or bacteria. Equally, it acts as a constant surveillance system, ensuring that damaged/abnormal cells are destroyed and cleared to maintain physiological homeostasis. In the context of tumourigenesis, the concept of cancer immunosurveillance has been extensively debated since it was first proposed in the late 50's, but it is now recognised that the immune system can indeed function to recognise and prevent primary tumour formation and contribute to the selection of immune evasive cancers through immune editing [1,2]. More recently, the complexity of the interaction between the tumour and the immune system, and the importance of the tumour microenvironment (TME), has become evident and it is now acknowledged that immune cells and inflammation play roles in tumour proliferation, migration and survival, representing a key hallmark of cancer.

GRUPPO DI QUESITI N. 2

1 In 10 minuti, spieghi ad una classe di liceo come si può utilizzare la PCR per quantificare i livelli di un mRNA specifico.

2 Descriva il progetto di ricerca di cui si è occupato/a nel corso della sua ultima esperienza in un laboratorio di ricerca.

3 Legga e traduca il brano in inglese presentato dalla commissione, tratto da DNA Repair vol 120:10349.

Brano in inglese: The tumour microenvironment is infiltrated with several types of immune cells, such as B cells, T cells and NK cells; and the immune response against tumour formation and progression is a result of competing inhibitory and stimulatory signalling pathways. Under normal circumstances, the immune system ensures that cells are protected against infection and tumour development, however tumour cells have evolved many strategies to evade immune surveillance. One of these strategies, is to upregulate the activity of inhibitory pathways by promoting the expression of negative regulators of the immune system, such as CTLA-4, PD-1/PDL-1, LAG3, TIM-3 and TIGIT, whilst inhibiting pathways/molecules that are responsible for stimulating the immune response, such as OX40, GITR, ICOS and CD40 [4]. Importantly, from a therapeutic

perspective, as these regulators are surface molecules, their activity can be easily inhibited through the use of antibodies that prevent ligand-receptor interactions, which has led to the development of several immune checkpoint blockade (ICB) therapies.

GRUPPO DI QUESITI N. 3

1 In 10 minuti, spieghi ad una classe di liceo i diversi sistemi di sequenziamento di acidi nucleici.

2 Descriva il progetto di ricerca di cui si è occupato/a nel corso della sua ultima esperienza in un laboratorio di ricerca.

3 Legga e traduca il brano in inglese presentato dalla commissione, tratto da DNA Repair vol 120:10349.

Brano in inglese: Another hallmark of cancer, genomic instability, is tightly linked with immune surveillance and editing, with the high frequency of coding mutations occurring in genomically unstable tumours leading to the production of proteins that are not normally expressed. These potentially serve as novel antigens (neoantigens) that may be detected by the immune system [6]. Such neoantigens can be derived from single nucleotide variants (SNVs), insertions and deletions (Indels), gene fusions, frameshift mutations and structural variants, and can be presented on the tumour cell surface, and/or by dendritic cells, via MHC complexes, following cancer cell death, driving immune activation (Fig. 1) [7]. Although tumour cells widely express neoantigens through major histocompatibility complex I (MHC-I), it has more recently been appreciated that expression of MHC-II restricted neoantigens, more commonly found on professional antigen-presenting cells, also play a key role in the anti-tumour response, as well as impact immunotherapy responses [8,9].

GRUPPO DI QUESITI N. 4

1 In 10 minuti, spieghi ad una classe di liceo come si può analizzare un estratto proteico per determinare il livello di una proteina di interesse.

2 Descriva il progetto di ricerca di cui si è occupato/a nel corso della sua ultima esperienza in un laboratorio di ricerca.

3 Legga e traduca il brano in inglese presentato dalla commissione, tratto da DNA Repair vol 120:10349.

Brano in inglese: Given the promising results obtained with immunotherapy in certain patients/tumour types, a plethora of studies have continued to focus on identifying reliable predictive biomarkers for response to immune checkpoint blockade therapy. PD-L1 expression on tumour epithelial cells and/or tumour-associated immune cells was among the first suggested biomarkers of response, with several studies indicating that its expression is predictive of response to anti-PD-1 therapy [13–15]. However, other studies have not found PD-L1 to be a reliable biomarker, as patients with no observed tumoural/immune PD-L1 expression can also benefit from anti-PD-1 therapy and vice versa [16–19]. Another widely assessed biomarker of ICB response is tumour mutational burden (TMB). Despite showing initial promising results in several tumour types, such as melanoma, lung cancer and urothelial carcinoma, subsequent studies have shown that in isolation TMB is not a reliable biomarker.

GRUPPO DI QUESITI N. 5

1 In 10 minuti, spieghi ad una classe di liceo come si può analizzare una preparazione di RNA mediante elettroforesi.

2 Descriva il progetto di ricerca di cui si è occupato/a nel corso della sua ultima esperienza in un laboratorio di ricerca.

3 Legga e traduca il brano in inglese presentato dalla commissione, tratto da DNA Repair vol 120:10349.

Brano in inglese: In addition to promoting tumourigenesis, DNA repair deficiencies can also represent a therapeutic opportunity to target cancer cells. The use of PARP inhibitors in homologous recombination (HR) defective tumours is an excellent example of how such defects can be exploited therapeutically [31,32]. Similarly, patients with mismatch repair (MMR) deficient colorectal tumours were the first to be shown to

have significantly improved responses to an anti-PD1 antibody (Pembrolizumab) [29]. Once established that other solid tumours with microsatellite instability/defective MMR were also susceptible to PD-1 inhibitors, the US Food and Drug Administration (FDA) approved the use of anti-PD1 therapy based on this biomarker, regardless of the histologic origin of the tumour, leading the way towards a treatment based on molecular profiling rather than tumour type [33]. Since then, DNA repair deficiencies have been extensively studied to identify possible associations between the DNA damage response (DDR) and response to immunotherapies.

GRUPPO DI QUESITI N. 6

1 In 10 minuti, spieghi ad una classe di liceo le tecniche principali di microscopia a fluorescenza.

2 Descriva il progetto di ricerca di cui si è occupato/a nel corso della sua ultima esperienza in un laboratorio di ricerca.

3 Legga e traduca il brano in inglese presentato dalla commissione, tratto da DNA Repair vol 120:10349.

Brano in inglese: In addition to the increased neoantigen load/TMB and TIL levels associated with DNA repair deficient tumours, the identification of a gene expression based molecular subtype of breast cancers, in which double strand break repair (DSBR) deficient tumours (enriched for BRCA1/2 mutant tumours) showed activation of the innate immune response, has shed light on an additional mechanism through which DDR deficiency (DDRD) may contribute to immune activation and response to ICB therapies [44]. This study, which aimed to identify a gene expression based molecular classifier for DNA repair deficient breast cancers, found that BRCA1/2 mutant breast tumours, as well as bone marrow from Fanconi Anaemia (FA) patients (harbouring mutations within various FA genes), could be identified via upregulation of innate immune signalling pathways, particularly genes classically associated with response to viral infection [44].

GRUPPO DI QUESITI N. 7

1 In 10 minuti, spieghi ad una classe di liceo come si possono studiare le interazioni proteina-DNA.

2 Descriva il progetto di ricerca di cui si è occupato/a nel corso della sua ultima esperienza in un laboratorio di ricerca.

3 Legga e traduca il brano in inglese presentato dalla commissione, tratto da DNA Repair vol 120:10349.

Brano in inglese: Interestingly, we and others have shown that in HR defective tumour cells, e.g. BRCA1/2 deficient cells, fragments of dsDNA, thought to be by-products of replication fork instability in a HR defective background, are exported to the cytoplasm, where they act as substrates for cGAS, leading to STING dependent innate immune activation [51–53]. Additionally, it has been shown that large chromosomal fragments induced either directly by DNA damage, or missegregated during mitosis as a result of persistent/unrepaired DNA damage, are encapsulated within micronuclei when the nuclear envelope reassembles after mitosis. cGAS accumulates within these micronuclei, leading to potent cGAS and subsequent STING activation upon micronuclei rupture, which can occur spontaneously or during a second round of mitosis [51–53]. In this context, intrinsic genomic instability leads to activation of the cGAS/STING pathway (Fig. 2). Additionally, cGAS/STING activation, and the associated interferon response, also leads to upregulation of immune checkpoint genes such as PD-L1, resulting in evasion of immune mediated tumour cell killing.

GRUPPO DI QUESITI N. 8

1 In 10 minuti, spieghi ad una classe di liceo come si possono studiare le interazioni proteina-proteina.

2 Descriva il progetto di ricerca di cui si è occupato/a nel corso della sua ultima esperienza in un laboratorio di ricerca.

3 Legga e traduca il brano in inglese presentato dalla commissione, tratto da DNA Repair vol 120:10349.

Brano in inglese: In order to prevent a disproportionate inflammatory response and avoid chronic inflammation, cells have evolved several mechanisms of protection. The most apparent and simple of these, is the containment of self-DNA within the nucleus, which prevents cytosolic cGAS from accessing and binding DNA and from consequently activating the inflammatory pathway. Nevertheless, it has recently been shown that cGAS can also be found in the nucleus [62]. In order to prevent cGAS activation by self-DNA in the nucleus and during mitosis when the nuclear membrane is degraded, several groups have demonstrated that cGAS binds tightly to histones 2A and 2B, which prevents its interaction with nucleosomal DNA and therefore inhibits its catalytic activity [63–65]. Additionally, it has been suggested that cGAS can also suppress DNA repair within the nucleus and promote tumour development, so it is possible that cGAS nuclear function might also modulate the innate immune system, however this requires further investigation [66,67].

Milano, 18 gennaio 2023

La Commissione

Prof. Muzi Falconi Marco - Presidente

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