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Perspectives

David versus Goliath: Radiotheranostic nanomedicine as a weapon against melanoma

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ABSTRACT

Malignant melanoma (MM), especially when diagnosed at an advanced stage, still represents a challenge for physicians. In recent years, immune check point inhibitors (ICI) have thoroughly changed MM landscape, although only 20–40% of MM patients respond to ICI. In MM progressing after ICI, treatment options, especially in case of MM not bearing V600 mutation, are limited. In this scenario, radionuclide theranostics, based on the sequential administration of a radiopharmaceuticals' pair, the first labeled with a radionuclide emitting energy suitable for imaging (i.e. positrons or gamma-rays), the second bound to another nuclide emitting particles for therapy, is particularly welcome. Melanocortin 1 Receptor (MC1R), strongly overexpressed by MM cells, has recently emerged as an interesting target for radionuclide theranostics. In the following, we briefly cover some emerging applications of MC1R-targeted radionuclide theranostics, also with reference to the potential of implementing some innovative nanotechnologies, such as gold nanoparticles, to move the field forward.

David versus Goliath: radiotheranostic nanomedicine as a weapon against melanoma

Malignant melanoma (MM) represents a leading cause of cancer-related death worldwide. The therapeutic landscape of MM has been thoroughly changed by the introduction of immune check point inhibitors (ICI) [1]. However, it has to be highlighted that only 20–40% of MM patients respond to ICI, therefore, especially in case of MM not bearing BRAF V600 mutation, therapeutic options remain still limited. In this scenario, the so-called "theranostic approach", entailing the administration of a radiopharmaceuticals' pair, one labeled with a radionuclide suitable for imaging and the other one bound to a radionuclide emitting alpha or beta particles aimed to exert a cytotoxic effect, is particularly welcome [2]. The first step to move forward a theranostic approach is represented by the identification of a suitable target, consisting in a molecular signature overexpressed by tumor and only minimally detectable in normal tissues. Furthermore, this tumor-associated biomarker should be preferentially located on cells' membrane, in order to have it exposed to an effective binding to the radiopharmaceutical utilized in diagnostic and therapeutic phases. The diagnostic phase is usually performed by utilizing an imaging agent labeled to a positron emitting radionuclide, in order to exploit the high performance of positron emission computed tomography (PET/CT) technology, or, alternatively, it can be bound to a gamma-emitter thus allowing imaging with gamma-camera and single photon emission computed tomography (SPECT). Subsequently, the same molecule utilized for the diagnostic phase, or a biosimilar agent, can be labeled with a particle-emitting radionuclide for therapeutic purposes. It has to be underscored that some radionuclides (i.e. ^{177}Lu, ^{188}Re, ^{64}Cu) emit both particles (alpha or beta) and positron or gamma-rays, therefore they can be used for diagnosis or therapy at different dosages.

In recent years, Melanocortin 1 Receptor (MC1R), a G protein-coupled receptor whose natural ligand is alpha-Melanocyte Stimulating Hormone (MSH), has emerged as potential target for theranostic applications in MM, as shown in **Fig. 1**. What makes MCR1 particularly suitable to the aim is that it is overexpressed in about 80% of melanomas and related metastases and, once bound to its ligands, it is internalized by tumor cells, resulting in a more effective tumor cell irradiation and lower organ toxicity [3].

The first theranostic studies exploiting MC1R receptor as a molecular target were performed on a population of B16/F10 melanoma-bearing mice by Yubin Miao et al., who developed several MCR1-avid peptides, belonging to the alpha-melanocyte–stimulating hormone's (alpha-MSH) family (i.e. CCMSH), labeled with different radioactive isotopes,

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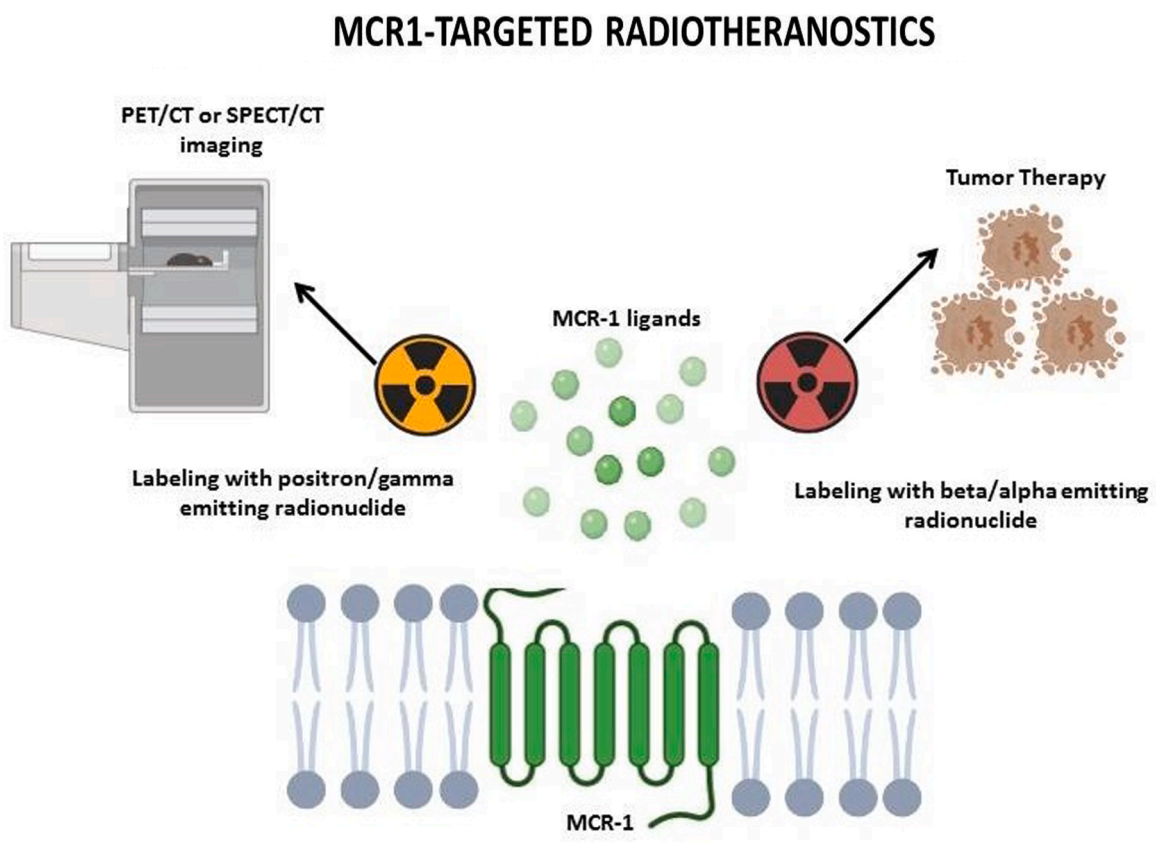

Fig. 1. Schematic representation of the different theranostic approaches directed toward melanocortin receptor-1, a melanoma-associated target (figure created with BioRender.com).

as follows:

a) ^{188}Re-(Arg11)CCMSH: ^{188}Re is a gamma- and also a high-energy beta-particle emitter, which allows irradiating different layers of tumor cells and is therefore ideal for large tumors [4].
b) ^{177}Lu- DOTA-GGNle-CycMSH$_{hex}$: ^{177}Lu emits gamma-rays with 2 photopeaks (113 and 208 keV) that are suitable for SPECT imaging, and it is also a medium-energy beta−particle emitter with a maximum soft tissue penetration of 1.8–2 mm, which is ideal for the treatment of small tumors and metastases [5]. In virtue of being gamma-emitters, both ^{188}Re and ^{177}Lu can be used to detect the best individual therapeutic dose *via* provisional dosimetry through scintigraphy/SPECT imaging.
c) ^{212} Pb- DOTA-GGNle-CycMSH$_{hex}$: ^{212}Pb is an alpha-emitter, which is suitable for treating small tumors and metastases due to high linear energy transfer (LET) and particles' short path length [6]. Furthermore, alpha-particles can induce clustered DNA damages, difficult to be repaired by tumor cells, regardless of cells' oxygenation status and cell cycle phase. However, while ^{177}Lu and ^{188}Re are both beta and gamma-emitters and therefore may be used both for imaging and therapy, ^{212}Pb is a pure alpha-emitter, so it cannot be used for imaging.

The biodistribution and tumor-targeting properties of all aforementioned radiopharmaceuticals were examined in B16/F10 tumor-bearing mice [7]: after radiocompounds' injection, all organs showed a rapid radioactivity clearance, except for the kidneys. Of note, the co-injection of a MC1R agonist (i.e. NDP-MSH) confirmed the receptor-mediated tumor uptake (i.e. reduction of the tumor uptake without influencing the kidney uptake). The aforementioned radiocompounds (i.e. ^{188}Re-(Arg11)CCMSH, ^{177}Lu-DOTA-*Re*(Arg11)CCMSH, and ^{212}Pb-DOTA-*Re*(Arg11)CCMSH, respectively) showed a relevant therapeutic effectiveness in animal models, since their administration led to a significant improvement in mean survival time in all treatment groups compared to the saline placebo control groups [7].

The introduction of a novel class of lactam bridge-cyclized alpha-MSH peptides (DOTA-GGNle-CycMSH$_{hex}$) led to an increased uptake of radioligands in MM cells and decreased kidneys' uptake, resulting in a lower renal toxicity, respect to peptides belonging to (Arg11)CCMSH's family. Studies have been performed with both ^{177}Lu-DOTA-GGNle- and ^{212}Pb-DOTA-GGNle-CycMSH$_{hex}$ [8]: since ^{212}Pb is a pure alpha-emitter, whose properties can be exploited for radiotherapy but not in the diagnostic field, it is possible to exploit the gamma emission of the ^{203}Pb isotope for the imaging *via* SPECT: ^{203}Pb, in fact, is a radionuclide with a 51.9 h half-life that generates 279 keV gamma-rays, suitable for SPECT imaging. Since ^{203}Pb/^{212}Pb share identical radiolabeling chemistry, this paves the ground to potential theranostic approaches [8]. Therefore, the combination of ^{203}Pb/^{212}Pb-DOTA-GGNle-CycMSH$_{hex}$ might be exploited for imaging-guided targeted alpha therapy in MC1R-positive MM patients.

MCR1-targeted radionuclide theranostic approaches can be combined with nanotechnology, for a more accurate delivery of the cytotoxic load (i.e. alpha or beta-particles) to the target. Furthermore, specific properties of some nanocarriers can be exploited to enhance tumoricidal effect. For example, gold nanoparticles, when properly stimulated with light at specific wavelengths, exhibit localized plasmon surface resonance (LPSR) that can result in photothermal and photoacustic effects [9].

Zhao et al. studied gold nanocages, functionalized with an alpha-MSH peptide, radiolabeled with the radionuyclide ^{64}Cu [10], which has physical properties particularly suitable for theranostics, since it is both a positron and beta-emitter and therefore can be used, at different

Table 1
Platforms for radionuclide-based theranostic approaches in malignant melanoma.

References	Radiocompound	Radionuclide Physical Properties	Diagnostic Platform	Therapeutic Platform
Miao et al. [4]	^{188}Re-alpha-MSH analog	^{188}Re Half-life 17.00 h Maximum beta energy: 2120.4 keV Gamma energy:155.0 keV	Gammacamera/ SPECT	Targeted beta-therapy
Guo et al. [5]	^{177}Lu- DOTA-GGNle-CycMSHhex	^{177}Lu Half-life: 6.647 days Maximum beta energy: 500 KeV Gamma energy: 113 and 208 keV	Gammacamera/ SPECT	Targeted beta-therapy
Yang et al. [8].	$^{212/203}$Pb-DOTA-GGNle-CycMSH$_{hex}$	^{212}Pb Half-life: 10.64 h Alpha daughters: ^{212}Bi (6.1 MeV) and ^{212}Po (8.8 MeV) ^{203}Pb Half-life: 51.873 h Gamma energy (EC decay): 279.2 keV	Gammacamera/SPECT	Targeted alpha-therapy
Zhao et al. [10]	^{64}Cu-AuNCs-PEG-MSH Nanoparticles (nanocages)	^{64}Cu Half-life: 12.7 h Positron energy (Eβ+) = 653.1 keV Maximum beta energy: 0.573 MeV	Positron Emission Tomography (PET/CT)	Targeted beta-therapy *plus* photothermal effect
Chen et al. [11]	^{89}Zr-DFOalpha-MSH-PEG-Cy5-C' dots	^{89}Zr Half-life: 78.42 h Positron energy (Eβ+) = 0.902 MeV	Positron Emission Tomography (PET/CT	Possible exchange of ^{89}Zr with beta or alpha emitter
Yang J et al. [13]	^{68}Ga-DOTA-GGNle-CycMSH$_{hex}$	^{68}Ga Half-life: 67.7 min Positron energy (Eβ+) = 1.899 MeV	Positron Emission Tomography (PET/CT)	Possible exchange of ^{68}Ga with beta or alpha emitter

dosages, for diagnosis and therapy. Mice bearing B16/F10 melanoma tumors were administered with alpha-MSH functionalized gold nanocages (i.e. ^{64}Cu-AuNCs-PEG-MSH) or with the same nanoparticles not bound to the alpha-MSH peptide. Functionalized nanocages showed significantly higher accumulation into tumors at microPET performed at 24 h, respect the not functionalized ones. Of note, the authors demonstrated that an increased coverage of nanocages with alpha-MSH peptides led to a further increased uptake of the nanoparticles within B16/F10 tumors. Both functionalized and non-functionalized nanocages showed high blood retentions at 4 h, followed by a rapid decrease at 24 h. These preliminary results suggest that nanocages functionalized with alpha-MSH peptides might represent an excellent multifunctional platform for theranostic applications: since they can be used for detection through PET/CT (thanks to ^{64}Cu's positronic emission), radionuclide therapy (thanks to ^{64}Cu's beta-emission) and photothermal loco-regional therapy. Once gold nanocages have been injected, in fact, they can be localized through PET/CT imaging and near infra-red laser (NIR) may be applied to the exact location to produce photothermal effect, thus combining photothermal heating and radionuclide therapy to eradicate cancer. Of note, ultrasmall silica nanoparticles, functionalized to target MC1R (i.e. alpha-MSH-PEG-Cy5-C' dots), have been recently introduced as multimodality platform for MM: after conjugation with the positron emitter ^{89}Zr, they have applied for imaging through PET/CT, showing favorable biodistribution and renal clearance kinetics in animal models [11]. Furthermore, alpha-MSH-PEG-Cy5-C' dots, labeled with alpha emitter ^{225}Ac, provided survival benefit in mice bearing B16/F10 melanoma tumors [12].

Although these preliminary results are intriguing and encouraging, MCR1-targeted theranostic nanomedicine is still far from passing "from bench to bedside". A cornerstone in this field has been represented by the first-in-human application of ^{68}Ga-DOTA-GGNle-CycMSH$_{hex}$, utilized for the PET/CT imaging of brain metastases in 2 patients affected by MM brain metastases [13]. There is still much to be learned in this field, especially as far as it concerns safety profile and long-term effects of the different radiotheranostic platforms (**Table 1**). Further studies need to be performed to assess whether or not radiotheranostic nanomedicine is going to represent a powerful and, hopefully, fatal weapon against MM in next future, just like David, smaller and apparently weaker, was capable to kill the giant Goliath with a well settled slingshot.

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Declaration of Competing Interest

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