UVEAL vs CUTANEOUS MELANOMA: ORIGINS AND CAUSES OF THE DIFFERENCES

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1. INTRODUCTION:

Melanoma is one of the tumours that showed highest increase rate in the latest decade in western countries **(1).** The highest rates worldwide are amongst fair skin population from Australia and New Zealand, where they reached epidemic proportions (Males: 50 cases/100.000 habitants /year, Females: 37/100.000) **(2).**

Melanomas of the ocular and adnexal structures comprises around 5% of all melanomas, and 85% are uveal origin **(3).** Uveal melanoma is the most frequent primary malignant intraocular tumor in adults. The tumour arises from melanocytes located in the iris, the choroid, and the ciliary body, which are the structures of the uveal tract. The reported incidence from several countries ranges from 4.9 to 10.9 per million population remaining stable for the last 50 years. Uveal melanoma is most commonly seen in an older age group of white population with light skin, hair and eyes, with a progressively rising age-specific incidence rates that peaks at age of 70 years**. (4-5).**

It usually presents as a pigmented choroidal nodular mass in the eye fundus, in early stages, growing towards the vitreous space, mushroom shape or multilobular with associated retinal detachment in advanced stages, an growing at the end towards the extraocular structures through the emissary channels or the optic nerve. Accuracy in diagnosing choroidal melanoma with indirect ophthalmoscopy and ultrasound by expert ocular oncologists has improved in last thirty years from 80% **(6)** to more than 99,5% **(7).**

Objectives for treatment include tumor destruction, preservation of the eye and the visual function, and survival improvement. Treatment options includes from observation (small, inactive tumors) to enucleation (large tumors without potential for visual recovery), but the most extended conservative therapy is episcleral brachytherapy or charged-particle radiotherapy. This techniques have proven to harvest excellent local control with a 5 years recurrence rate less than 5%, and survival rates similar to enucleation **(8, 9)**

Although significant advances have been made in the ability to diagnose and treat the primary uveal melanoma, they have unfortunately not been accompanied by improvements in the survival rate of the patients. Mortality rates have not changed in the last decades and reach more than 40% of patients developing systemic disease and dying within 10 years of diagnosis **(10).** This fact suggest that the disease was already disseminated at the time of diagnosis **(11)** either as circulating malignant cells (CMCs) **(12)** or as occult micrometastatic lesions **(13)** Unfortunately, current clinical tests fail in detecting metastatic disease at an early stage **(14).**Once the disease has metastasized, life expectancy is reduced to <1 year **(15)** Treatment options for patients with clinically disseminated disease are scarce and usually unsuccessful

The treatment of intraocular melanoma has evolved recently. Enucleation has been superseded largely by brachytherapy, proton beam radiotherapy, stereotactil irradiation, transcleral local resection, transretinal resection and diode laser phototherapy **(16).** Principal diagnostic parameters are tumour diameter, age and sex of the patient, together with the histology and localization of the tumour **(17).**

2. CAUSES OF THE DIVERSITY OF THE MELANOMAS

Caucasian race seems to be the most significantly affected, with light skin color, blond hair, and blue eyes, being specific risk factors to oncogenic effects of sunlight.

Ultraviolet light have been involved on the genesis of several cutaneous melanomas **(18).** It has been suggested that melanomas were due to an intense and intermittent exposure to sunlight, that do not leave time for melanocytes to synthesise melanin protect them from the ultraviolet irradiation (UV), producing DNA mutations. Although there are evidences that support this hypothesis in skin melanoma, the evidence in regard to uveal melanoma is insufficient and contradictory **(19).**

Although they are both of neural crest origin, significant differences can be found between cutaneous and choroidal melanocytes and cutaneous melanoma derives from melanocytes that migrates to the epithelia **(20)** while uveal melanoma derives from melanocytes that migrates deeply to mesodermic tissues **(21).** A better molecular understanding of these cellular populations could help to understand the variability on the behaviour and response to therapies of both melanomas **(22).**

In this context, cell cycle associated proteins has been purposed but other parameters such as the stem cell origen, immunological differences etc have been suggested **(23).** We will go onto some of these hypothesis in this review.

2.1. MELANOMA STEM CELLS.

The cancer stem cells (CSC) hypothesis opened new perspectives for the treatment of tumours. CSC have been identified in several malignancies by type–specific cell surface markers often associated with stem cells **(24-27)**..

There are two proposed genetic origins for cancers **(28)** ***Figure 1***. The stochastic model, predicts that every cell within a tumour can form new primary tumours, and this mechanism forms the basis of most tumour therapies. By contrast, the hierarchical or stem cell model predicts a rare subset of cells as tumorigenic. The tumour is heterogeneous in appearance, consisting of cells at different degrees of differentiation.The discovery that bone marrow-derived stem cells home to sites of tissue damage **(29-30)** opens up a third possibility for the origins of cancer. Normal stem cells could migrate to the tumour and fuse with mutated somatic cells, giving rise to immortal, malignant cancer stem cells **(31).** This hypothesis can explain both the heterogeneity of this kind of tumours and their variable responses to several conventional therapies ***(Figure 2).***

MELANOCYTIC STEM CELL MIGRATION TO DIFFERENT AREAS

Melanomas are believed to arise from a mature, differentiated melanocyte. With regard to cutaneous malignancies, increasing evidence supports the stem cell theory in the pathogenesis of squamous cell carcinoma and malignant melanoma. In this scenario, a transformed, differentiated melanocyte may have undergone dedifferentiation and regained stem cell properties such as self-renewal **(32).** Melanocytes found in the skin and in the choroid layer of the eye derive from the neural crest, a transitory structure formed at the dorsal borders of the neural plate during development of vertebrates. Melanocytic precursors, melanoblasts (which themselves are unpigmented but have the potential to produce melanin) invade all skin areas and differentiate into melanocytes. How neural crest cells become committed to the melanocytic lineage for eye or skin and which are the factors controlling survival, proliferation and differentiation of melanocyte precursors are still not completely clear.

The analysis of mouse white spotting mutants allowed to the identification of stem cell factor (SCF, also denominated as steel factor) and its tyrosine kinase receptor c-Kit as crucial components of a pathway required for survival and migration of pigment precursors cells **(33).** Other studies characterized the implication of endothelins **(34),** in particular endothelin-3, to be required together with SCF/c-Kit for migration of early melanoblasts in the dermis **(35).** Another pathway likely to be involved in melanocytic migration is Wnt/beta-catenin that controls neural crest cell fate and also activates Mitf expression **(36, 37).** In fact, Fang and colleagues (**38),** using a combination of Wnt3a, endothelin-3 and SCF, were able to derive melanocytes from human embryonic stem cells. These melanocytes were able to migrate to the epidermal basal layer in reconstituted skin.

STEM CELL MARKERS

Like many types of cancer, the treatment of cutaneous and uveal melanomas could benefit tremendously from the identification of specific tumour stem-cells markers. But the scarcity of these cells within the tumour mass (that comprise > 99.9% of most cancers) make this, a severe technical challenge. In addition, many tumour stem cell markers are probably shared with normal stem cells. Tumour specific patterns of gene expression, designed to allow the tumour-stem cell to survive outside its protective “niche” in normal tissues, will be the best initial targets for new therapeutic agents.

These and other studies demonstrated that aggressive melanoma cells share many characteristics with embryonic progenitors. Stem cells have a complex relationship with their microenvironment which exerts a crucial role in all stages of tumorigenesis including initiation, progression and metastasis **(39).** Lastly, a number of laboratories have shown that an embryonic microenvironment has the capacity to reverse the metastatic phenotype of cancer cells. Thus, several studies have documented that embryonic microenvironments reprogram aggressive melanoma cells towards a less aggressive phenotype **(40).** Furthermore, these studies have uncovered Nodal, an embryonic morphogen belonging to the TGF-beta family, as an important factor for sustaining melanoma aggressiveness and plasticity **(41).**

ATTEMPTS TO ISOLATE CANCER STEM CELLS IN MALIGNANT MELANOMA.

A population that fullfils the criteria for melanocyte stem cells was identified by Nishimura and co-workers in the lower permanent portion of mouse hair follicles **(42).** However, it is unknown whether this cell population is multipotent or restricted to the melanocytic lineage. Interestingly, a population of multipotent adult stem cells, that gives rise to differentiated smooth muscle cells, neurons and melanocytes, has been isolated from human hair follicles **(43).**

CSCs from brain tumours could be isolated, and proliferate as nonadherent cell aggregates termed “spheres or spheroids” **(44).** It has been speculated that the association between tumours of the nervous system and malignant melanomas in certain individuals represents an underlying abnormality in neural crest stem cells. In 20% of the metastatic melanomas cultured in growth medium suitable for human embryonic stem cells, a subpopulation of cells propagating as nonadherent spheres was found, whereas in standard medium, adherent monolayer cultures were established. Individual cells from melanoma spheres (melanoma spheroid cells) could differentiate under appropriate conditions into multiple lineages, such as melanocytic, adipocitic, osteocytic, and chondrocytic lineages, which recapitulates the plasticity of neural crest stem cells **(45).**

Recently, in other studies, the ability to efflux Hoest dye was utilized in another attempt to isolate cells with stem cell-like features **(46).** These cells also expressed the stem cell-associated surface marker CD133 (as the cancer stem cells in brain tumours), and the chemoresistace mediator ABCB51. In serial human-to mouse xenotransplantation experiments, ABCB51 melanoma cells possess greater tumorigenic capacity than other populations and re-establish clinical tumour heterogeneity. In vivo genetic lineage tracking demonstrates a specific capacity of ABCB51 subpopulations for self-renewal and differentiation. Identification of melanoma-initiating cells with enhanced abundance in more advanced disease but susceptibility to specific targeting through a defining chemoresistance determinant has important implications for cancer therapy. All these data should be tested to determine whether or not are applicable to uveal melanoma.

2.2. GENETICS IN UVEAL MELANOMA:

As a result, new prognostic factors in uveal melanoma have been described that also serve as molecular targets for the development of novel treatments. These prognostic factors/molecular targets, such as membrane receptors, enzymes, cytokines, cytoskeleton components, oncogenes, tumour suppressor genes, cell-cycle proteins and nuclear antigens. The recent advent of DNA micro-array technology now offers an unprecedented ability to study these molecules and others associated with malignant transformation.

Data from several transcriptome analysis **(47)** suggest that at least three main molecular pathways have been found frequently dysregulated in melanoma tissues. These pathways include the Ras-Raf-MEK-ERK pathway.

Oncogenic RAS maintains cells in a proliferative status and enhances the expression of tumour cell adhesion molecules, but it is also a powerful activator of the PI3K-AKT pathway **(48).** This pathway controls cell cycle progression **(49),** provide strong survival signal **(48)** and regulate crucial cellular functions including adhesion; angiogenesis, migration and resistance to drug treatment **(50).**

It was found that cutaneous melanoma often shown frequent mutations in BRAF (60% of cases) and NRAS genes (20% of cases) **(51, 52)** suggesting that mutations in BRAF gene may be necessary but not sufficient to promote melanoma development**.** However, BRAF and NRAS mutation are extremely rare in uveal melanoma **(53).**.But chromosome deletion of locus 10q harbouring PTEN gene **(54)** has been observed in 30-50% uveal malignant melanoma **(55)** that could contribute to the constitutive activation of the PI3K-Akt pathway in this cancer type.

Observation that the $p16^{INK4A}$-CDK4-RB tumour suppressor pathway is frequently inactivated in cutaneous melanoma at intermediate stage **(56)** suggest that dysregulation of theses two pathway may cooperate to provide crucial biological functions required for the metastasis process. In contrast to the well-described genetic alterations found in cutaneous melanoma, not much is known about genes associated with development of uveal melanoma, mainly because of low abundance of biopsies samples accessible from patients developing this disease.

However it has been demonstrated **(57, 58)** that uveal melanomas develop characteristic chromosomal abnormalities, such as loss of chromosome 3, abnormalities in chromosome 6 and at a lesser frequencies gains in chromosome 8q. This is associated with a reduction in the 5-year survival from approximately 95% to less than 50% **(58).**

2.3. UVEAL MELANOMA AND IMMUNE RESPONSE

Melanomas of the eye have the advantage of growing in the special environment of an immune privileged site and it has long been shown, that the special immunosuppressive properties of the intraocular microenvironment are essentially mediated by cytokines.

The immune surveillance hypothesis was introduced over 30 years ago **(59)** and it proposes that neoplasms express novel antigens that subjected them to immune detection and elimination. In order for immune surveillance to be effective in controlling neoplasms, the tumor must arise in a body site that permits the induction the full array of immune responses The unique immunologic and anatomic features of the eye prevent the induction and expression of conventional immunity-a phenomenon known as 'immune privilege'. Although ocular immune privilege represents a theoretical obstacle to immune surveillance. The presence of either tumor-infiltrating lymphocytes (TIL) or tumor-infiltrating macrophages (TIM) is associated with poor prognosis in uveal melanoma patients **(60)** and suggests that some immune responses to intraocular tumors might exacerbate, rather than mitigate, tumor progression.

3. CLINICAL RESPONSE IN UVEAL MELANOMA

Certain natural properties of melanoma stem cells are likely to increase the tumour resistance to standard chemotherapy agents. Since cancer stem cells are believed to be more resistant to our current chemotherapeutics, allowing them to persist and regenerate tumours, understanding these different expression patterns will be of paramount importance in producing more effective treatments. Therefore, in developing new cancer therapeutics, the toxicity towards tumour stem cells is an important priority **(61).** Thus, if cancer therapies do not effectively target the cancer stem cell population during initial treatment, relapse may occur.

Uveal melanoma is characterized by constitutive chemoresistance. The chemoresistance of uveal melanoma is mainly due to the typical multidrug resistance phenotype (MDR), which is linked to overexpression of membrane proteins that actively extrude anticancer drugs from the cell. Typical MDR is particularly complex in this tumor since several chemoresistance-related proteins are simultaneously produced **(62).** The negative prognostic significance of the overexpression of P-glycoprotein, the main representative among the typical MDR-related proteins, was shown in uveal melanoma. The atypical MDR phenotype, which refers to other chemoresistance mechanisms such as resistance to apoptosis also contributes to the chemoresistance of uveal melanoma.

CONCLUSIONS.

Even with advances in the diagnosis and local treatment of uveal melanoma, there has been no significant change in the survival rates of these patients in the last decades. Metastatic disease still occurs at the same frequency, and no systemic therapy is currently offered to patients after local eye treatment. As a result of these limitations, there is interest in gaining a greater understanding of molecular changes associated with aggressive disease patterns in uveal melanoma. This might result in new, more effective and less toxic therapies as well as provide prognostic information for defining subgroups of patients with a less favourable prognosis as potential candidates for adjuvant therapies.

. Therefore, since a stem cell population have been isolated in cutaneous melanoma. there is hope to isolate the one may exist in uveal melanoma. This may provide new targets for treatments.

Thanks to the recent progress in molecular biology, the chemosensitization strategies of gene therapy approaches, which aim at weakening the pathological activity of MDR genes in cancer cells, are currently on the rise. From the complexity of signalling involved in carcinogenesis, it is clear that each cancer is unique in the set of signals and group of cells involved in its development. Therefore, are to be developed newer more effective treatments for cancer, several obstacles must first be overcome and further studies need to be done.

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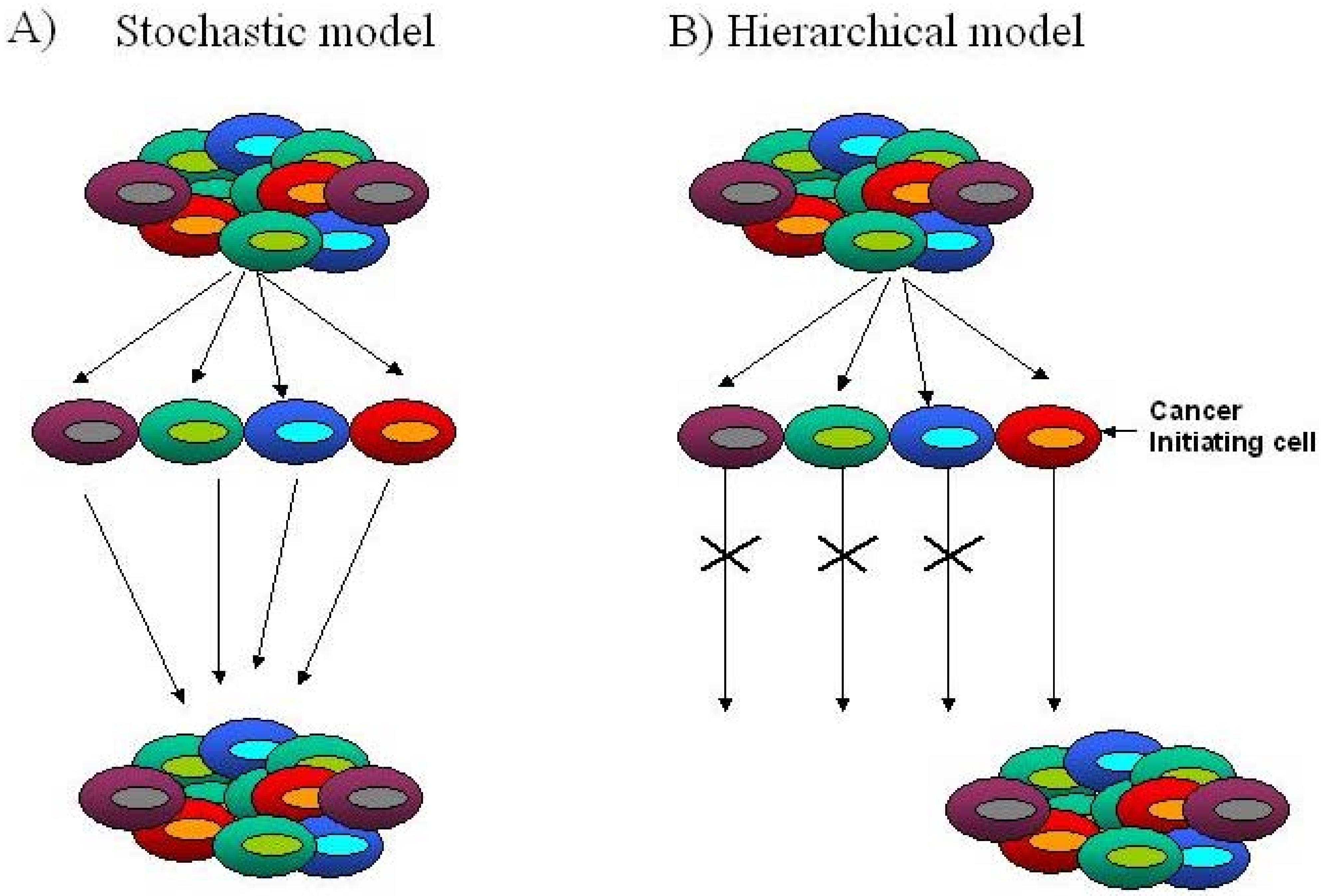
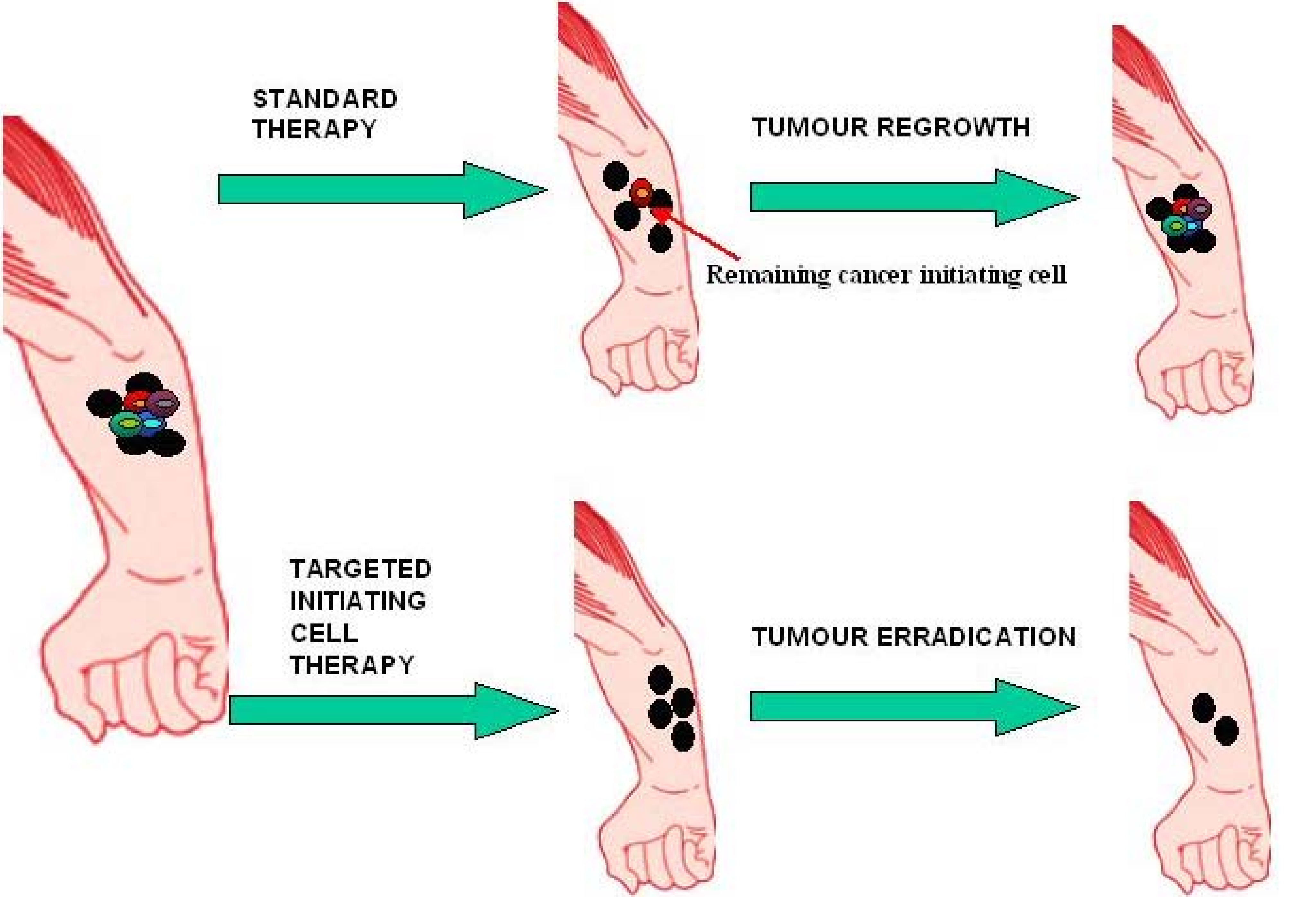
LEGENDS OF FIGURES

FIG 1. Stochastic versus hierarchical model of tumour formation. In the stochastic model of carcinogenesis, cells form a heterogeneous tumour from which each cell is able to randomly give rise to new carcinomas. In the hierarchical model of carcinogenesis, just a few cells (<5% of tumour mass), known as the cancer stem cell (CSC) are able to rise new tumors.

FIG 2. Cancer stem cells targeted therapies: Cancer stem cells are often the population which shows resistancy to chemotherapy. The lack of efectivity against these cells is sufficient to allow regrowth of the tumor. In the new cancer stem cell targeted treatment model, elimination of these CSCs may prevent tumor recurrence.


A) Stochastic model
B) Hierarchical model
Cancer
Initiating cell


STANDARD THERAPY
TUMOUR REGROWTH
Remaining cancer initiating cell
TARGETED INITIATING CELL THERAPY
TUMOUR ERRADICATION