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Perspective

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Boudewijn van der Sanden*[Δ], Florence Appaix*, François Berger*[Δ], Laurent Selek*, Jean-Paul Issartel* and Didier Wion*[Ω].

***INSERM U836, Grenoble Institut des Neurosciences, Université Joseph Fourier, CHU Michallon, Grenoble, France ; [Δ]CLINATEC, Centre de Recherche Edmond J. Safra, MINATEC Campus, CEA, Grenoble, France.**

Ω corresponding author : didier.wion@ujf-grenoble.fr

running title: The cancer cell trap concept.

Abstract: Viewing tumors as ecosystems offers the opportunity to consider how ecological concepts can be translated to novel therapeutic perspectives. The ecological trap concept emerged about half a century ago when it was observed that animals can prefer environment of low quality for survival over other available of higher quality. The presence of such a trap can drive a local population to extinction. The cancer cell trap concept is the translation of the ecological trap into glioma therapy. It exploits and diverts the invasive potential of glioma cells to guide their migration towards specific locations where a local therapy can be delivered efficiently. This illustrates how an ecological concept can change therapeutic obstacles into therapeutic tools.

Key words: *Ecological trap; glioma; cancer therapy.*

Executive Summary

The ecological trap concept

An ecological trap occurs when environmental cues lead animals to migrate and settle in poor quality habitat where they experience lower survival or reproductive success.

In agriculture, the ecological trap concept has led to the development of attract-and-kill techniques that combine for example a pheromone with an insecticide for the local eradication of pest animals.

The cancer cell trap concept, a two-step therapy

The cancer cell trap therapeutic paradigm is the translation of the “attract to kill” strategy to cancer therapy.

Cancer cells are first attracted by non-toxic stimuli towards a therapeutic field where lethal radiation doses are then delivered.

Future perspectives

The cancer cell trap concept is a conceptual and technological therapeutic challenge exploiting and directing cancer cell migration properties.

Combining the “attract to kill” and “search and destroy” approaches in a unifying paradigm will probably be one of the next breakthroughs in cancer therapy

Introduction.

It is now widely accepted that tumors can be considered as ecosystems in which cancer cells, host cells and the extracellular matrix interact together [1]. Accordingly, cancer cells behave like animal species do in an ecosystem. They compete for space and resources, reshape their environment, are subjected to predation by the immune system and spread into their tissue of origin or migrate to colonize other organs. Considering cancer as an ecological process provides the opportunity to consider how concepts of ecology can provide new therapeutic perspectives [1,2].

Despite decades of research and clinical trials, and more than “60 000 publications” indexed in PubMed, glioma remains among the most deadly tumors. The disease usually progresses through brain invasion, with glioma cells still detectable several centimeters away from the core lesion. The infiltrative nature of glioma cells is a major cause for tumor relapse as it limits the long term efficiency of therapeutic approach targeting the tumor mass such as brain surgery and radiotherapy (Fig. 1). The containment of the tumor growth in brain that can be considered as a single ecosystem, suggests that glioma could be a valuable model for investigating how some concepts issued from ecology could be transferred to cancer therapy.

The ecological trap concept.

The concept of ecological trap emerged more than forty years ago [3] (Fig. 2A). An ecological trap occurs when a low-quality habitat is preferred over other available habitats of higher quality [4-8]. Ecological traps occur when a novel element in the environment mimics a traditional cue for habitat choice, thereby misleading the animals [5]. Importantly, an ecological trap can drive a local population to extinction [6]. An example of an ecological

trap is provided by the effect of light pollution on sea-turtles hatchlings [4]. When sea-turtle hatchlings emerge at night from their sand nests, they move directly toward the brightest direction which normally corresponds to the open horizon and therefore to sea (Fig. 2B). However, this sea-finding behavior is disrupted when human light sources behind the beachfront provide a super-normal stimulus that cue hatchlings to migrate inland where they die (Fig. 2B). Importantly, the stimulus is not toxic per se but acts as a misinformation cue. Another example is provided with asphalt track and waste oil lakes. Such structures have water-imitating polarized light which attract polarization-sensitive water-seekings insect with the consequence that eggs laid on such surfaces inevitably perish [7]. Again, the ecological trap results from a mismatch between traditionally attractive cues and the actual quality of the habitat [5,6]. The principles of ecological trap are of major concern for the conservation of animal populations, but they have also been used for the local eradication of pest animals. Some examples of behavior modifying compounds used in such attracticide approaches include artificial light or pheromone traps to lure pest species to a location containing insecticide.

Translation of the ecological trap concept to glioma therapy.

Infiltrative growth is a pathognomic feature of malignant cells and often causes cancer relapse, metastasis and consequently death. Regarding glioma, extracranial metastases are rare, and in most cases it is the existence of cancer cells in the brain but outside the core lesion that makes complete surgical resection impossible and limits other local therapies such as radiotherapy. Chemotherapy also failed to eliminate disseminated cancer cells, and all attempts to increase drug delivery have not provided major breakthrough. Hence, in cancer, tumor relapse and patient death are the consequence of our impossibility to eradicate invasive or metastasic cancer cells. Consequently much effort is dedicated to

developing anti-invasive therapies [9,10]. A potential limitation of such anti-invasive approaches is that the development of invasive cancer cells can be an early event during tumor progression [10,11]. For example, in glioblastoma, cancer cells have already moved away from the tumor core at the time of diagnosis and even hemispherectomy have not ensured eradication of the disease [10]. By analogy with the ecological trap concept and with the attracticide technologies developed for pest species, an alternative to anti-invasive therapy would be to exploit the invasive potential of glioma cells to guide their migration in order to concentrate them towards specific locations where a local therapy could be delivered efficiently (Fig. 2C). Rather than inhibiting cancer cell invasion and migration, the originality of this attracticide approach exploits and diverts the invasive potential of cancer cells themselves for a therapeutic purpose. Attracticide technology also represents an alternative to the conventional delivery of chemotherapeutic drugs. Since cancer cells will target the site where drug is located, highly effective concentrations can be achieved locally while reducing the total amount used compared to conventional therapies. This also provides an alternative to conventional radiotherapy by the possibility to focus X- or γ-ray microbeams to the sites where attractors or traps are implanted. Moreover, since only migrating cells are attracted and contact the trap, the attracticide therapy preserves both the tissue environment and normal non-targeted cells. Importantly, the cancer cell trap must be viewed as a part of an integrated therapy for the prevention of tumor relapse as a complement to therapies that shrink the primary tumor mass.

The cancer cell trap.

The cancer cell trap concept requires combining a cancer cell attracting field with a local chemotherapeutic and/or radiotherapeutic approach in a single therapeutic device (Fig. 2C). After treatment or resection of the solid tumor mass, residual migrating cancer cells are

attracted into a site where a cytotoxic therapy is then delivered. This “attract to kill” strategy can be considered as a two-step therapy. It first requires attracting cancer cells. Directed cell migration plays important roles both in physiological and pathological processes such as host defense, wound healing and cancer. In addition biology gradients of concentration guide cells and determine cell fate during embryogenesis [12]. In the case of gliomas, invasive glioma cells follow myelinated fibers and blood vessels within the perivascular spaces that are less densely packed with extracellular matrix [9,13]. Basically cell migration can be directed by chemical concentration gradients (chemotaxis), gradients of immobilized surface molecules (haptotaxis) or physical stimuli such as electric stimuli (electrotaxis). Several neurotransmitters have been shown to have chemoattractive function towards cancer cells [14]. Nevertheless, the systematic screening of the chemoattractive potential of neurotransmitters towards glioma cells remains to be done. More data are available regarding chemoattractive cytokines. For example, neural progenitors are attracted by TNFα or IFNγ towards the site of injection where inflammation occurs [15]. Malignant glioma cells express several other chemoattractant receptors such as FPR1, CXCR1, CXCR2, CX3CR1 [16]. Another chemotactic factor for glioma cells is hepatocyte growth factor (HGF) [17]. Convection enhanced delivery (CED) can be used to improve the diffusion of such chemoattractants in the brain [18,19]. It is noticeable that drug delivery can generate concentration gradients which limit the efficiency of anticancer drugs [20]. However, in the case of chemoattractive molecules, this limitation in the drug delivery process is exploited as it will generate the chemoattractant gradients necessary to direct cell migration. A notable point is that many chemoattractive cytokines are also involved in cancer progression since they induce for example a motile phenotype. Obviously, the therapeutic use of factors known to be involved in tumor growth for cancer therapy seems counter-intuitive. However,

this paradox must be overcome as it corresponds to the rationale of an ecological trap *i.e.* a super-normal non-toxic stimulus that cues organisms to migrate into a place they die. In the cancer stem cell paradigm, only a subpopulation of cancer cells drive cancer growth and tumor relapse. Determining which chemokines are chemoattractive for cancer stem cells is therefore critical. In addition to chemical gradients it is recognized that endogenous direct-current electric fields also provide directional cell migration in a process named electrotaxis [21-28]. This guided cell migration is also observed for stem cells [26-28]. A direct current electric field of strength 250 mV/mm induces the cathodal migration of neural precursor cells but has no effect on their differentiated progeny [27]. Electric fields also direct migration of lung, breast and prostate cancer cells and the degree of electrotaxis is more significant for invasive cancer cells [24,25,29-31]. Just like pest control in fields depends on the optimal position and density of attracticide point sources, the location of tumor traps in brain patients will be critical. Since glioma relapse mostly occurs within 2cm around the resection cavity [10], implanting the tumor trap in the tumor bed after resection and around migratory tracks is a first option. Regarding the delivery of the cytotoxic agent, local delivery of drugs such as carmustine with for example wafer implants (Gliadel®) in the postoperative tumor bed are currently used. The modest increase in survival for patients [32] is probably due to the low diffusion distance of this drug from the wafer into the brain that is limited to few millimeters [32,33]. One critical point regarding the low penetration of BCNU is that because it is liposoluble it goes into bloodstream through capillary walls before diffusing to any appreciable distance [34,35]. On the other hand, high molecular weight molecules such as IgG whose diffusivity is nearly 10^6 smaller than that of BCNU, shows much greater penetration depth of nearly 2.2 cm compared to BCNU (0.5 cm) [34]. Therefore the generation of gradients could be paradoxically easier with high molecular weight and water

soluble proteins than with low liposoluble molecules. Although, drug penetration can be enhanced by convection delivery (CED) [18,19], the systemic toxicity required for targeting disseminated cancer cells with efficient drug concentrations enlightens the limitation of this kind of interstitial therapy approach if it is not combined with a cancer cell attracting field. However, because of glioma stem cell chemoresistance, stereotactic radiosurgery with high dose delivery is probably the most efficient therapy for targeting trapped cancer cells. Recent developments in new radiotherapy protocols have shown that a high radiation dose can be applied locally in one shot sparing normal adjacent brain tissue using synchrotron-generated X-ray beams ('microbeams') or gamma radiation [36-38]. The radiation cytotoxicity may be enhanced considerably if radiosensitizing agents are present in the cancer cell trap [39]. The cancer cell trap approach should also lead to reevaluate the efficiency of stereotactic brachytherapy whose current limited efficiency is probably related to cancer cell diffusion in the brain parenchyma [40]. Attracting and concentrating cancer cells in the trap location should allow the use of permanent iodine-125 low-activity implants limiting the risk of brain necrosis [41].

The technological challenge

A therapy based on the ecological trap strategy will have to overcome several steps before it becomes a useful therapeutic tool. The final step for eradication of cancer cells once they have been concentrated into the therapeutic field can be managed with the now available improved means for irradiation. Indeed, ongoing advances in stereotactic radiosurgery such as microbeam radiation therapy are able to deliver with high-precision a radiosurgical therapeutic dose to a target volume of 7 mm^3 while sparing surrounding tissues [37]. Probably the most challenging part of the cancer stem cell trap strategy is the generation of a cancer stem cell attracting field *in vivo*. A first step will be to determine the chemotaxis or

electrotaxis parameters capable to guide cancer cell migration in the brain. Experimental procedures for tracking the migration of implanted cells in the rodent brain are available. For example, modified neural stem cells (NSC) expressing either firefly luciferase or any fluorescent protein can be tracked in the rodent brain by bioluminescence imaging or multiphoton microscopy [42-45]. This approach has successfully demonstrated the extensive capacity of NSC to migrate towards sites of cerebral pathology in the rodent brain [42-45]. These *in vivo* imaging technologies can be used to determine which migratory cues are effective to guide implanted cancer cells to specific locations in the brain. Regarding the generation of migratory chemotaxis gradients in the brain, a device that can configure such gradients in the human brain is not currently available but could be developed. Microfluidic devices controlling flow rates, concentrations and release sequence could be combined with CED to establish spatial pattern of chemoattractive agents [46,47]. Likewise, combining stereotactic injection with CED is another option for creating chemical gradients [48]. The creation of chemical gradients *in vivo* will also benefit of the current progress in the field of drug delivery. Surface properties of drug-loaded microparticles can be engineered to interact more or less with extracellular matrix [49]. Using a mixture of chemoattractant-loaded microparticles with different bioadhesive properties could be a way to generate concentration gradients and to combine chemotaxis with haptotaxis. In comparison to chemotaxis, electrotaxis has less been studied even if the use of a direct-current electric field for guiding cancer cell migration has been successful in vitro [24,25,29-31]. Consequently, our knowledge on the mechanisms and on the therapeutic potential of electrotaxis is still in its infancy [50]. Currently, research on the therapeutic application of electrotaxis mainly focuses on its potential for guiding cells in stem cell therapy or tissue engineering [50-53]. Regarding the cell specificity of these attractive cues, we have to consider that every cell in

the brain with a high migratory potential could be affected by a therapeutic attracting field. In the adult brain, cell migration mainly concerns immune and neural stem cells. Stem cell niches in the adult brain are localized in the subgranular zone of the hippocampus and in the subventricular zone [54]. Attracting fields should avoid these locations and a set of parameters preferentially attracting cancer stem cells should be determined first.

Once the cancer cell trap concept is validated on animal models, then the efficiency of this "attract and kill" strategy can be evaluated on patients after tumor resection. Assessment of treatment response will benefit of the combination of advanced magnetic resonance imaging (MRI) and position emission tomography (PET) [55-57]. Diffusion Tensor Imaging and fluid-attenuated inversion recovery (FLAIR) are two MRI techniques that measure changes in the apparent diffusion of water induced by cancer cell invasion or concentration [55-57]. In addition to MRI, PET imaging can be used to detect the highly localized concentration of cancer cells in the trap and to assess the response to radiosurgery. The glucose metabolism (^{18}F-2fluoro-2-deoxy-d-glucose (FDG)), amino acid uptake (^{11}C-methionine (MET) or cell proliferation (^{18}F-fluorothymidine (FLT) which all have been shown to be increased in glioma cells can be evaluated at the trap site. The amount of ^{18}F-FDG correlates with the tumor cell density, ^{11}C-MET and ^{18}F-FLT PET are superior for the detection of respectively infiltration zones and cell proliferation zones with high sensitivity and specificity [55-57] Finally, overall patient survival will represent the end-point for measuring clinical efficacy.

Perspectives.

Cancer therapy since its beginning has been directed by a “search and destroy” strategy in which cancer cells are the targets. However the invasive nature of this disease, and in the case of glioma its diffusive growth, suggest the interest of a complementary “attract and kill” approach. The physician and philosopher of science Canguilhem stated “To act, it is necessary at least to localize” [58]. Inspired by the concept of ecological trap, the cancer cell trap concentrates and localizes cells into a predefined targetable therapeutic field. To achieve this goal the invasive potential of cancer cells is exploited and diverted for a therapeutic purpose. In this regard, recent evidence suggests that the glioma cell invasive potential can be increased by current anti-angiogenic therapies [59]. Combining the tumor trap concept with an anti-angiogenic therapy could be an option to tackle this therapeutic side effect. Likewise, another therapeutic challenge, the existence of drug decreasing concentration gradients which limit chemotherapy efficiency [20], is exploited in the cancer cell trap approach to generate attractive chemotaxis gradients. Hence, the cancer cell trap concept by turning therapeutic obstacles into therapeutic tools can be viewed as an adaptive response to cancer cell invasiveness, and tumor relapse. Combining the “attract to kill” and “search and destroy” approaches in a unifying therapeutic paradigm will probably be one of the next breakthroughs in cancer therapy.

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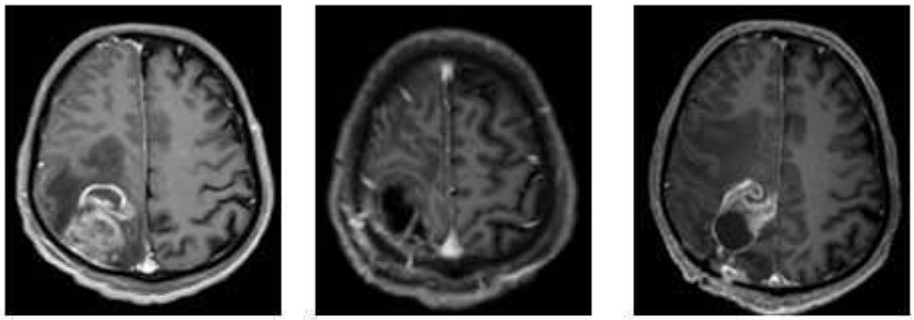

Fig.1. Local recurrence of a right parietal glioma.

A: Contrast enhanced magnetic resonance imaging scan at the time of diagnosis

B: post operative scan showing gross total resection

C: Recurrent tumor adjacent to the resection cavity.

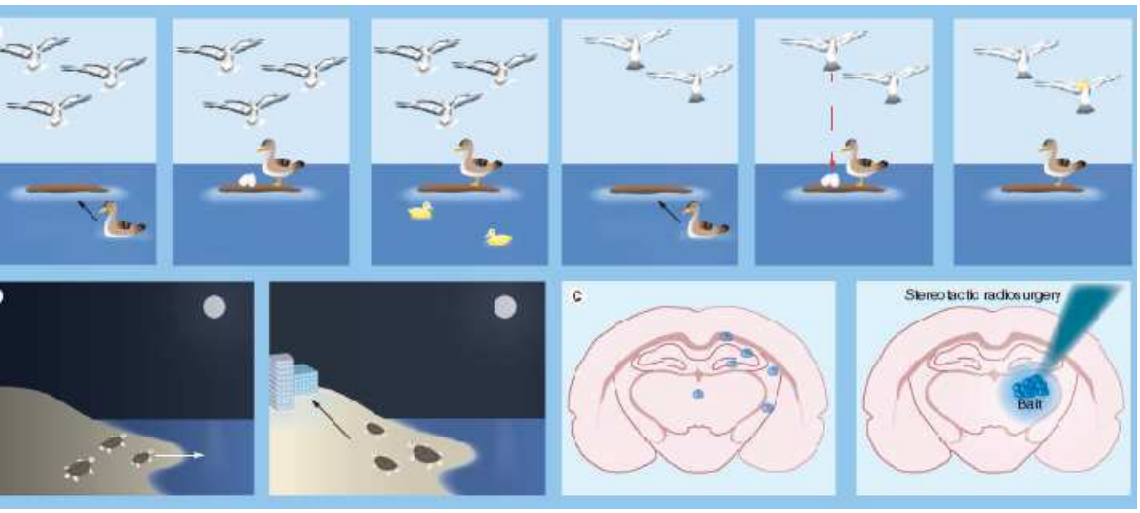

Fig 2. Natural, anthropogenically-induced and therapeutic ecological traps.

An ecological trap arises when a low quality habitat is made more attractive than a high quality habitat by a novel element in an environment that mimics a traditional cue for habitat choice.

A: ***Natural ecological trap:*** The concept of ecological trap was proposed by Dwernychuk and Boag forty years ago as a possible explanation for the observation that ducks and gulls nest in close association on islands in inland lakes although gulls attack ducklings [3]. In their

interpretation they suggest that terns, which do not attack ducklings, were the first larids to occupy these islands, providing protection against other nest predators. The later incursion of gulls onto these islands ended the successful relationship between ducks and larids, and generated an ecological trap.

B*: Anthropogenically-induced ecological trap:* For hatchling sea turtles emerging nocturnally, moonlight acts as a stimulus for sea-finding. Disruption of this sea-finding by human photopollution leads to the wandering of turtles to inland where they die [4]. Light or pheromone trapping of pest insects is an application of the anthropogenically-induced ecological trap principle.

C: *Therapeutic ecological trap:* Invasion of glioma cells throughout brain parenchyma is a major cause of therapeutic failure. The cancer cell trap approach exploits the ecological trap principles by diverting the cancer cell migratory potential for a therapeutic purpose. In the proposed example, the cancer cell trap is implanted in the tumor bed after surgery and attracts residual cancer cells. Thereafter, high radiation dose is delivered at the location where residual cancer cells have been concentrated. (purple circles: migrating cancer cells).

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