METHODS

An emerging technique for the dual-targeting chemo-radio-ablation of generic micro-cancers in conjunction with cancer liquid biopsy

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Abstract. Cancer liquid biopsy (CLB) aims for the detection of circulating tumor cells, cell-free DNA or RNA, and specifically expressed proteins, lipids and metabolites present in the bio-fluids of patients. Rapid advances in technologies for sampling such bio-specimens and for genomic sequencing have fostered the development of authority-approved CLB tests in clinical practice for the early screening and diagnosis of malignancies. However, perhaps some solid tumors could have been reliably detected using CLB, while they were still too small to be found in patients using currently available imaging technologies. This could impose troublesome clinical scenarios. In this communication article, the author would like to propose a potential solution that eventually may be used to combat such unwanted embarrassing clinical issues by using one episode of targeted radiotheragnostics, namely OncoCiDia.

Introduction of cancer theragnostics

Cancers, particularly solid tumors, remain a global concern posing a severe threat to human health (1). Immense efforts have been directed at exploring new technologies for the diagnosis and treatment of cancers in laboratory experiments and clinical practice, among which theragnostics is the newly emerging realm referring to a single targeting medication that enables both therapy and imaging diagnosis typically involving radiopharmaceuticals as a major player in the armamentarium of modernized precision medicine (2). This novel domain has even aroused public debates on whether to include the letter 'g' in this new term; the portmanteau word 'theragnostics' is more lexicologically justified (3).

Cancer liquid biopsy with possible drawbacks

As regards diagnosis in experimental and clinical oncology, cancer liquid biopsy (CLB) presents an innovative rapidly advancing high technology. Conventional histopathology remains the clinical gold standard for cancer diagnosis, of which invasive tissue biopsy is potentially risky, with limited sample accessibility and narrowed images for the entire heterogeneous tumor profiles. On the contrary, CLB presents a non-invasive or minimally invasive technique for the early detection of circulating tumor-derived components from the patient (4,5).

As shown in Fig. 1, for CLB, the biofluids from the blood, cerebrospinal fluid, saliva, pleural effusion, urine etc. are sampled and processed to identify circulating tumor cells, subcellular structures such as tumor-specific exosomes, circulating micro ribonucleic acid, circulating tumor deoxyribonucleic acid, and genomic and transcriptomic alterations including gene fusions, point mutations, methylations etc. CLB is useful for the screening, diagnosis and profiling if cancers, monitoring therapies and following-up patients, as well as for detecting tumor residuals and predicting prognoses. Current progress regarding CLB suggests that it may have an impact on the clinical outlook of several tumor types in the near future (4,5). However, despite increasing evidence supporting CLB as a valuable oncological tool (4,5), certain issues such as method sensitivity and substance scarcity or instability etc., still need to be resolved in order for CLB to be implemented as a clinical routine. For instance, plausible discrepancy between positive CLB outcomes and not yet available clinical countermeasures could lead to complex scenarios, i.e., in the case

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Abbreviations: CA4P, combretastatin 4 phosphate; VDAs, vascular-disrupting agents; CLB, cancer liquid biopsy

Key words: cancer liquid biopsy, micro-cancers, pan-anticancer, targeted radiotherapy, OncoCiDia chemo-radio-ablation, radiotheragnostics

that the super-sensitive CLBs clearly indicate the existence of malignant tumors in patients, but the cancers are too small to be detected by currently available imaging modalities (i.e., micro-cancers). This then may alarm, confuse or dishearten patients and their families, instead of helping or comforting them. Therefore, it is to address such a possible CLB-related clinical issue that a potentially revolutionary solution has been proposed in the present brief overview article. One of the aims of the present study was to promote multi-institutional verifications on this straightforward approach with collective expertise and infrastructure.

OncoCiDia as a novel anticancer theragnostic strategy

To combat solid cancers, a small molecule dual-targeting pan-anticancer theragnostic strategy has been elaborated to exploit the power of the natural cancer targetability (6,7), as acronymized using 'OncoCiDia' where Onco- stands for cancers, -Ci- for killing and -Dia for imaging diagnosis (8). This aims to offer a safe, simple, workable, affordable and generic solution for diverse types of cancer, and warrants further exploitation. The mechanisms of action for OncoCiDia are illustrated in Fig. 2 by the simulation of a liver cancer (Fig 2A). OncoCiDia consists of two sequential systemic drug-deliveries accomplished only within one episode of two consecutive days. On day 1, a cancer-targeting drug termed vascular disrupting agent (VDA), represented by combretastatin A4 phosphate (CA4P) that was originally discovered from African bush willow with a broad native anticancer spectrum, is intravenously injected to induce massive tumor ischemic necrosis (Fig. 2B and C) by the selective shutdown of tumoral blood vessels via depolymerizing the defected endothelial tubulin cytoskeleton (9).

However, VDA-induced tumor necrosis is only partial and always leaves residual viable cells behind for cancer relapse (Fig. 2B), which hinders the efficacy and authority approval of VDA, despite advanced clinical trials (9). Nevertheless, such tumoral necrosis forms an ideal target for the second targeting compound termed hypericin (Hyp), which is naturally extracted from St. John's wort (Fig. 2E) and also chemically synthesizable with several prior known medicinal applications such as anti-depression, antivirus, photosensitivity, etc. However, Hyp has been newly found with a strong necrosis-avidity with exploitable novel utilities (6,10). Thus, on day 2, the tumouricidal iodine-131 (^{131}I) is radiochemically labeled onto Hyp to form an ^{131}I-conjugated necrosis avid tracer (^{131}I-Hyp), which is then intravenously infused to selectively localize in and strongly bind to VDA-induced necrosis, while emitting high-energy β^--particles with 2-mm penetration and an 8-day decaying half-life to exert constant eradiation on remaining cancer cells by inducing their DNA damage (Fig. 2D and F). Moreover, its gamma rays facilitate scintigraphy imaging (Fig. 2G), hence being truly a theragnostic strategy. The non-necrosis-bond ^{131}I-Hyp can be eliminated via the hepatobiliary pathway, particularly when aided by certain safety measures, such as nasobiliary drainage (11,12). Since both the targets, namely abnormal tumoral vasculature and intratumoral necrosis, are naturally occurring and generic to all solid cancers, OncoCiDia has proven to be a truly pan-anticancer strategy (8).

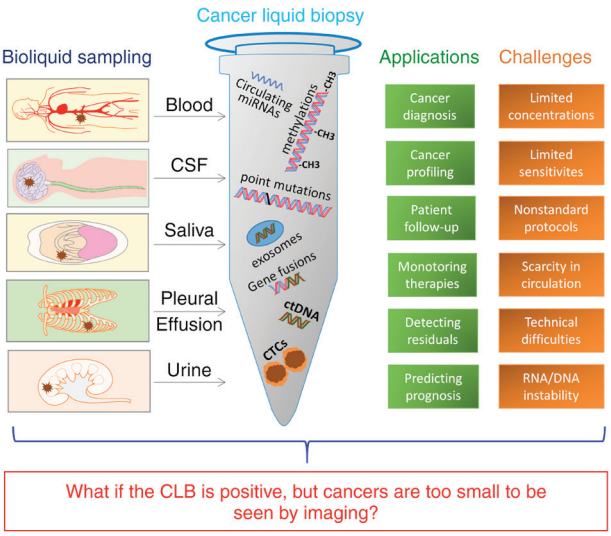

Figure 1. Schematic diagram of cancer liquid biopsy with applications and challenges.

Internal radiotherapy using radioiodine (^{131}I) is known to be curative for the majority of thyroid cancers, even at their metastatic stages (13). OncoCiDia was actually intended to extend such an excellent efficacy to the treatment of virtually all solid malignancies, due to its unique dual targetability and complementary synergy. The anticancer efficacy of systemically administered internal radiotherapy relies on the cumulative radiation dose in the target tumor. The potent targetability of ^{131}I-Hyp to the VDA-induced tumoral necrosis in OncoCiDia could render a therapeutically required cumulative radiation dose >50 Gy (6,7), consistent to that shown with curative treatment of differentiated thyroid cancers by iodine-131 (13). Currently, early clinical trials of OncoCiDia have been ongoing among both veterinary (https://www.dierenartsenwereld.be/nl/nieuws-n2/ugent-zoekt-honden-met-kwaadaardige-tumoren-i171/; accessed on November 28, 2022) and human (OncoCiDia Phase 0 study (3M150468; https://www.kuleuven.be/onderzoek/portaal/#/projecten/3M150468?hl=en&lang=en; accessed on December 22, 2022) patients, with curative potentials revealed in patients after only one episode of OncoCiDia, e.g., in one patient with a massive inoperable esophageal squamous cell cancer. Thus, OncoCiDia can most likely solve the bottleneck issues of the unmet therapeutic demand common for all those VDAs currently under preclinical and clinical developments.

Serendipitous findings indicative of a potential cure for early-stage cancers

Although having exhibited extraordinary anticancer potential, OncoCiDia is still considered largely as a palliative care for patients with late-stage disease. However, as demonstrated in Fig. 3, in recent studies, particularly among those on hypovascular and avascular micro-cancers (Fig. 3A1-A4 and B1-B4), it has been serendipitously found that CA4P caused almost complete necrosis in the majority of micro-cancers in millimeter scales (Fig. 3C1-C5 and D1-D5) (14,15), which is contradictory to what has been known in the literature

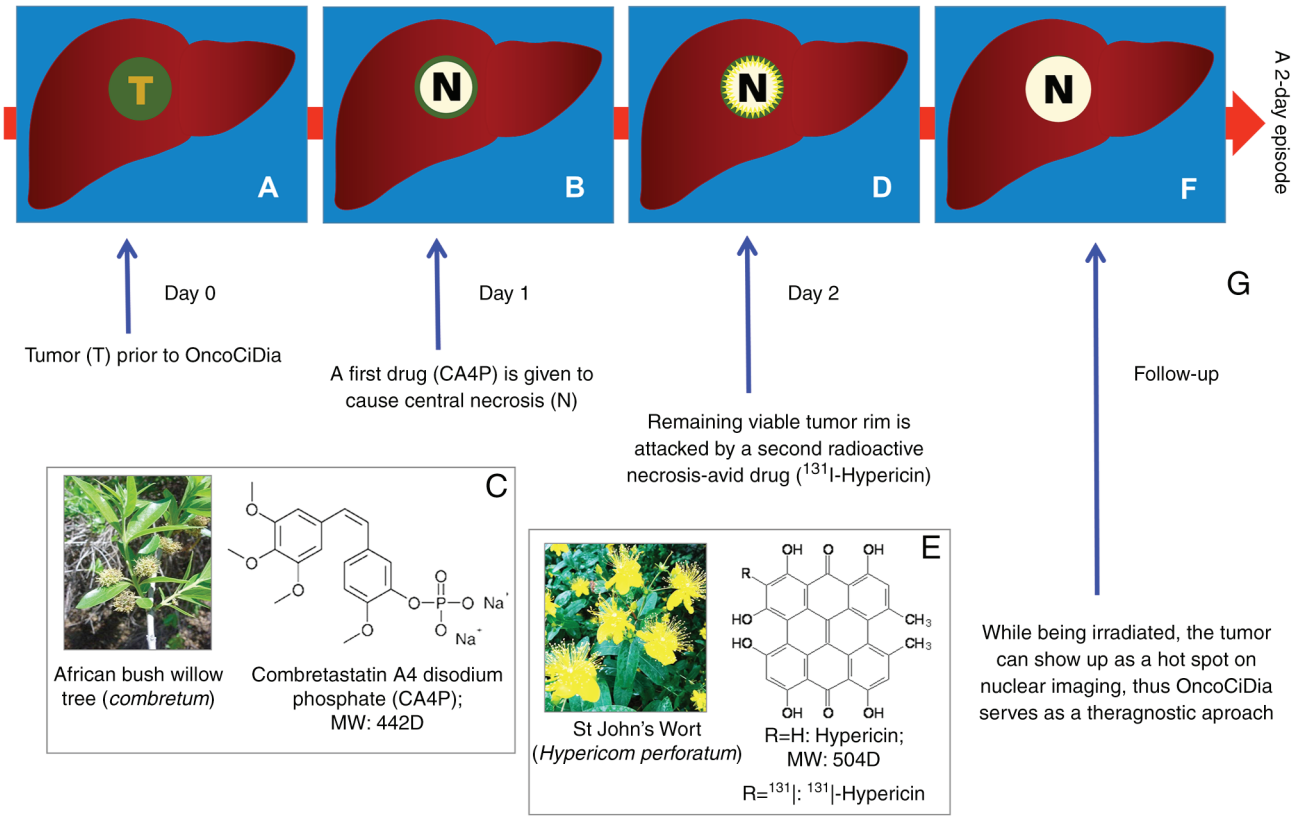

Figure 2. Schematic diagram of OncoCiDia as a dual-targeting pan-anticancer theragnostic strategy. In the case of (A) an unresectable tumor T in the liver, (B) on day 1, the tumor is largely necrotized through an intravenous injection of (C) CA4P to form a target (N) for the following treatment; on day 2, iodine-131 is conjugated onto Hyp and ^{131}I-Hyp (E) is intravenously infused to specifically localize in the newly formed necrosis (N), where (D) the residual viable tumor tissues are persistently irradiated by high energy β particles in a crossfire manner to (F) pursue cancer treatment; the ^{131}I-gamma rays facilitate scintigraphy imaging to inspect the tumor in real-time under OncoCiDia treatment (G); hence, a dual targeting multifunctional pan-anticancer strategy. A part of the images has been adapted from a previous study by the authors (7).

regarding VDAs, i.e., improved VDA therapeutic effects were positively associated with the increasing tumor volume with a larger amount of abnormal tumoral vasculature as the VDA target (16). It now appears that those immature vascular structures of CD34 positively stained endothelium (Fig. 3B5, C5 and D5) play a crucial role in sustaining the growth of very early stage solid cancers, which in turn can be effectively targeted and destroyed by VDAs too, leading to massive proportions of intratumoral necrosis with merely few layers of viable cancer cells left at the tumor periphery (Fig. 3C1-C5 and D1-D5) among the observed micro-cancers that are beyond the resolution of clinical imagers.

All naturally occurring malignant tumors undergo the avascular-hypovascular-hypervascular steps of cancerous angiogenesis according to Folkman's theory (17), and tumor vascularity can be defined by the density of tumoral blood vessels or intensity of tumor blood perfusion, relative to those of the host tissue or organ, as measured by different imaging modalities (18). Accordingly, almost all micro-cancers appear avascular or hypovascular (Fig. 3A1-A4) (14,15). However, Hori *et al* (19) made an artificial LY80 micro-tumor model by seeding tumor tissue into a prior-implanted transparent chamber to better observe in vivo tumor growth and drug reaction. However, the earlier chamber-surgery had already stimulated angiogenesis and thus speeded up the vascularization process of the later implanted tumor tissue, leading to a strange phenomenon of hypervascular micro-tumors, as observed in the studies by Hori *et al* (19) and Maeda (20) studies on the related topics. Therefore, such an artificial tumor model is not relevant for the interpretations of drug reactions in the natural avascular and hypovascular micro-tumors, and cannot help to predict the efficacy of VDAs among true avascular and hypovascular micro-tumors in animals (14,15) and humans.

The aforementioned experimental findings suggest that the generally palliative OncoCiDia could become a curative dual-targeting chemo-radio-ablation means with which to eradicate all solid cancers, particularly at their infancy or 'micro-tumor' stages (15,21).

Rationale of OncoCiDia for eradicating micro-cancers with the combined use of CLB

Given the aforementioned clues that: i) CLB may help detect the presence of solid malignancies that may be too small to identified in the body, which may trouble clinical management; and ii) micro-cancers may well respond to VDAs with possible curative outcomes once adjuvantly treated with ^{131}I-Hyp, it can be hypothesized that sub-centimeter cancers could be cured blindly in animals and humans by OncoCiDia under serial CLB surveillance, as coined by an international patent application (21). A possible scenario may occur in which a CLB confirms positive result for a certain type of cancer that

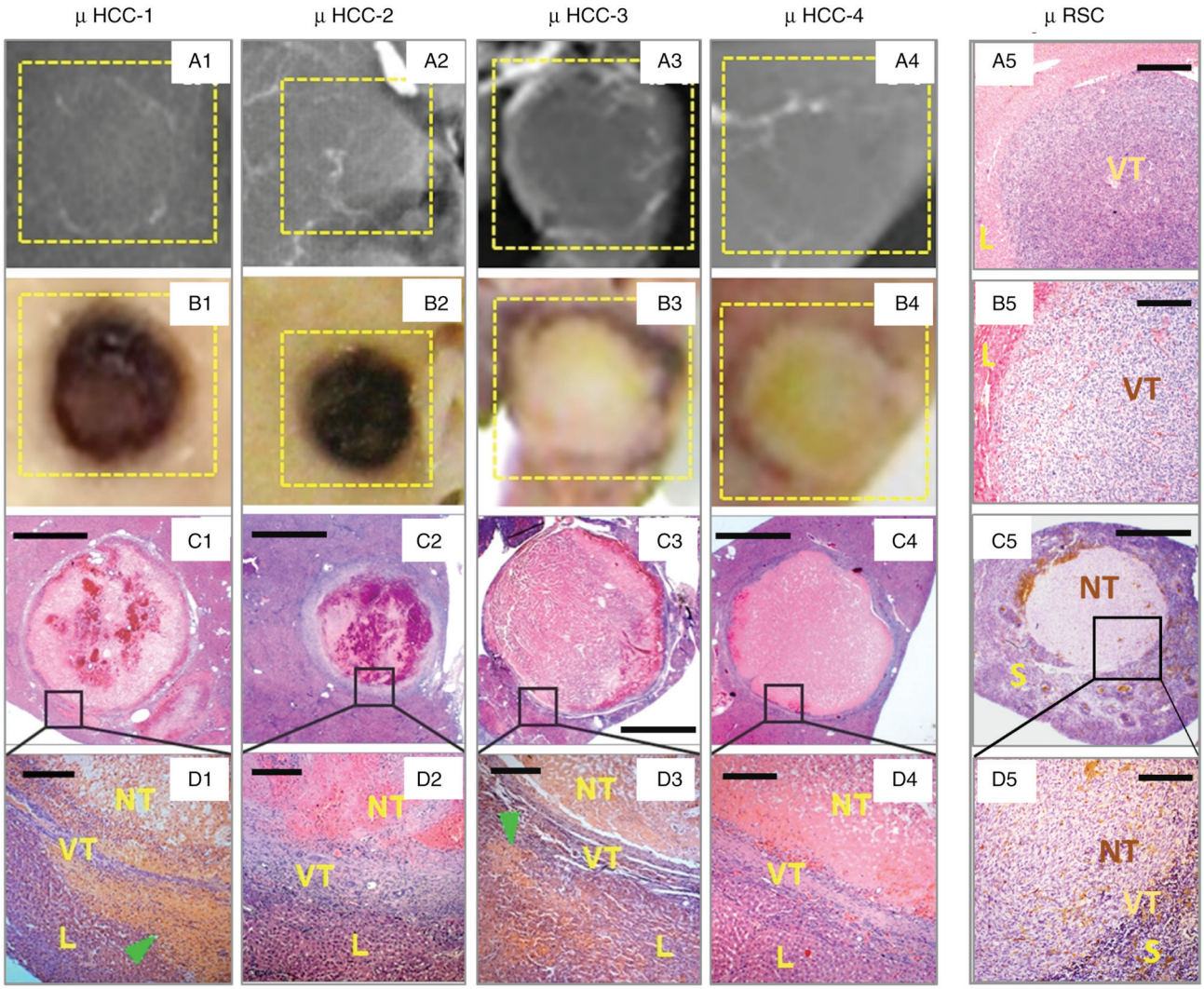

Figure 3. Incidental findings of surprising VDA efficacy in hypo- or avascular micro-cancers. Typical rat cases with (A1-A4) digital radiographic, (B1-B4) macroscopic, (C1-C5) low magnification and (D1-D5, and A5 and B5) high magnification views of micro-hepatocellular carcinoma (μHCC-1 to μHCC-4), and a micro-rhabdomyosarcoma (μRSC) from an untreated control rat with liver implantation following (A5) hematoxylin and eosin staining and (B5) endothelial transmembrane glycoprotein immunohistochemistry or CD34 staining, and from a VDA-treated rat with the splenic implantation of μRSC following (C5) hematoxylin and eosin and (D5) CD34 staining, as previously described (14). In general, these micro-tumors are (A1-A4) hypovascular or avascular, exhibiting typical VDA-induced (B1 and B2) hemorrhagic or (B3, B4 and C5) coagulative necrosis, as (D1-D5) microscopically proven, (D1-D4, A5, B5, C5 and D5) lacking apparent structural tumor blood vessels, (B5, C5 and D5) but presenting a positively stained intratumoral endothelium. L, liver; VT, viable tumor; NT, necrotic tumor; S, spleen. (C1-C5) Scale bar, 1 mm; (D1-D5, and A5 and B5) scale bar, 50 μ. Some images have been adapted from a previous study by the authors (14).

fails to be detected by even a whole-body PET/CT scan from a patient who is then subjected to an episode of OncoCiDia; a few weeks later if a follow-up CLB yields a negative result, this patient has likely been cured of that cancer. To verify this hypothesis, a geometric model has been established by considering multiple factors of pharmacology, radiophysics, radiobiology and mathematics, as illustrated in Fig. 4.

In Fig. 4, the left column is assigned for tumors before therapy, where a large (upper) and a small (lower) sized thorny sphere 1 simulates a macro and micro tumor (T), respectively with an invasive growth pattern characteristic of malignant tumors. The middle column refers to the same tumors following treatment with a VDA, which is known to induce massive tumor necrosis (TN) denoted by the inner sphere 2 with a viable shell (δ) at tumor periphery. The right column simulates the same tumors that are further treated by the intravenous administration of ^{131}I-Hyp that accumulates in the central necrosis (TN), particularly at the dead-alive interface, and emits high energy β particles that penetrate to ~2 mm in depth, which may be shorter (<) than the thickness of the viable tumor shell (δ), leading to a largely palliative effect in macro-tumors, but can be surely long enough (>) to cover the entire remaining cancer cells in all micro-tumors, achieving virtually total cancer cure. The detailed geometric deductions are illustrated in the lower part of Fig. 4 with the estimated curability of OncoCiDia among both micro- and macro-cancers shown in Fig. 5 of a colored comparative table where all micro cancers with >50% VDA-induced necrosis would be cured, which however have to be first validated by ongoing animal experiments, then replicated by other research groups, and eventually further confirmed by clinical trials in cancer patients.

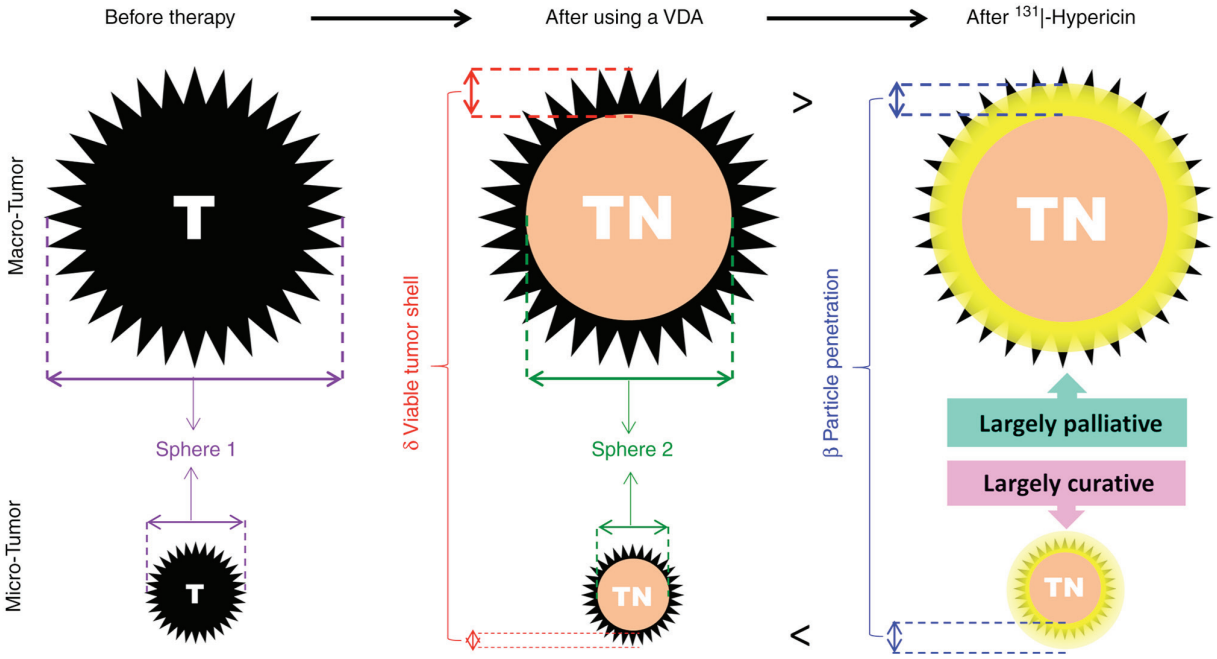

Figure 4. Geometric deduction of OncoCiDia for potentially curative outcomes among micro-cancers versus macro-cancers with largely palliative outcomes. VDA, vascular disrupting agent; T, tumors; NT, VDA-induced tumor necrosis; δ, viable tumor shell. β penetration, 2 mm. The volume of the sphere 1 (T) of radius r1 was calculated using the following formula: $V_1 = \frac{4}{3}\pi r_1^3$; the volume of the sphere 2 (NT) of radius r2 was calculated using the following formula: $V_2 = \frac{4}{3}\pi r_2^3$; suppose V2 is α% of V1 (e.g. 0.8 = 80%): $V_2 = \alpha V_2$; that is $\frac{4}{3}\pi r_2^3 = \alpha\frac{4}{3}\pi r_1^3$; thus $r_2^3 = \alpha r_1^3, r_2 = r_1\sqrt[3]{\alpha}$; the thickness of viable tumour shell δ will be: $\delta = r_1 - r_2 = r_1 - r_1\sqrt[3]{\alpha} = r_1(1-\sqrt[3]{\alpha})$. VDA is known to induce 50-99% (α) of tumour necrosis. Upon the calculations with the aforementioned formulae, in theory, only 10% of macro-tumors of 10 cm in diameter may achieve curative efficacy by OncoCiDia; however, nearly all micro-tumors <2 cm in diameter could be eradicated by using OncoCiDia only once.

Estimated OncoCiDia curability considering tumor size, VDA-necrosis rate and β emitting

cm / mm / %	Micro-cancers				Macro-cancers							
	0.2	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
99	0.003	0.008	0.015	0.030	0.045	0.060	0.075	0.090	0.105	0.120	0.135	0.150
90	0.035	0.088	0.175	0.350	0.525	0.700	0.875	1.050	1.225	1.400	1.575	1.750
80	0.072	0.180	0.360	0.720	1.080	1.440	1.800	2.160	2.520	2.880	3.240	3.600
70	0.112	0.280	0.560	1.120	1.680	2.240	2.800	3.360	3.920	4.480	5.040	5.600
60	0.157	0.392	0.785	1.570	2.355	3.140	3.925	4.710	5.495	6.280	7.065	7.850
50	0.206	0.515	1.030	2.060	3.090	4.120	5.150	6.180	7.210	8.240	9.270	10.03
40	0.263	0.658	1.315	2.630	3.945	5.260	6.575	7.890	9.205	10.52	11.84	13.15
30	0.331	0.828	1.655	3.310	4.965	6.620	8.275	9.930	11.59	13.24	14.90	16.55
20	0.415	0.208	2.075	4.150	6.225	8.300	10.38	12.45	14.53	16.60	18.68	20.75
10	0.536	1.340	2.680	5.360	8.040	10.72	13.40	16.08	18.76	21.44	24.12	26.80
0	1.000	2.500	5.000	10.00	15.00	20.00	25.00	30.00	35.00	40.00	45.00	50.00

Figure 5. Estimated OncoCiDia curability considering tumor size, VDA-necrosis rate and β emission. The top row presents the diameters of the assumed spherical tumors from 0.2 to 10 cm where micro-cancers are ≤2 cm in diameter and the remaining are macro-cancers. The left column presents the percentage of VDA-induced tumor central necrosis ranging from 10-99%. The bottom row is the control without VDA, of no effect. Based on the equation $\delta = r_1(1-\sqrt[3]{\alpha})$, the figures contained in this colored table are the thickness δ in mm of the viable rim leftover from VDA treatment, calculated by the radius of the tumor r_1 and the percentage of the VDA-induced tumor necrosis α, which defines a curative potential (pink) and palliative effects (green) based upon the penetration depth about 2 mm from β particles emitted by ^{131}I-Hyp. If there is no necrosis (α = 0), no therapeutic effects (δ=r_1) would be expected (grey). VDA, vascular-disrupting agents; Hyp, hypericin.

Distinguishing primary from metastatic tumors poses currently unsolved experimental and clinical issues, not to mention differentiation between micro-tumors of metastatic and primary origins, which are all under intensive multidisciplinary studies (22-26). However, the simple use of OncoCiDia combined with CLB to systemically eliminate all hypo- or avascular micro-tumors (whether primary or metastatic) may help to circumvent such complex cancer problems.

Conclusive remarks

Complementary to the newly emerging high technology of CLB, a novel technique OncoCiDia for the dual-targeting chemo-radio-ablation of generic micro-cancers is proposed by exploiting the natural existence of pan-anticancer targetability, efficacy and simplicity. First, this approach closely follows the natural rules of cancer pathology and mechanisms: i) All solid cancers rely on the proliferated aberrant neovasculature for supporting their growth, which leaves a specific defect for therapeutic intervention by VDAs (8); ii) as recently reported, those immature 'prevascular' endothelial structures observed in hypovascular and avascular micro-cancers can also be attacked by VDAs (14,15) (Fig. 3); and iii) tumor necrosis occurs both therapeutically and spontaneously in all solid cancers, which can be targeted by necrosis-avid agents that had been upgraded into radionuclide theragnostics (6-8). Secondly, this approach involves small molecular therapeutic elements, such as CA4P, Hyp and iodine-131 that are originally derived from the nature with unlimited economical resources and favorable safety profiles if applied properly (6-16,). As demonstrated by the preclinical modelling in the present study (Figs. 4 and 5), the combination

of CLB and OncoCiDia may bring about an alternative cancer cure, particularly at an early imaging-undetectable stage, which could imply a paradigm shift in oncology and warrants further investigations to substantiate and optimize its efficacy, safety and applicability. Safety-wise: i) CA4P in multiple doses has undergone advanced clinical trials (9,16), whereas only a single dose is needed for OncoCiDia; ii) nearly a micro-dose of Hyp is used in OncoCiDia with negligible chemotoxicity; and iii) the same dose of iodine-131 has been used for over the last half-century in the treatment of thyroid cancer, hence a relatively safe profile with OncoCiDia.

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Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Author's contributions

YN has made sole contributions to the conception, design of the work, the acquisition, analysis, and interpretation of data, has drafted the manuscript and revised it, made the decision to submit this manuscript, and will be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, are appropriately investigated, resolved, and the resolution documented in the literature. The author has read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate.

All animal experiments were carried out in compliance with the European and national regulations after obtaining approval from the KU Leuven Ethics Committee for Animal Care and Use, with particular codes P161/2014 and P046/2019, respectively.

Patient consent for publication

Not applicable.

Competing interests

The author YN is a sole inventor of a pending patent application as cited in ref 21 of this manuscript. The host institute KU Leuven, Belgium, to which he belongs, is the IP owner of this patent application, which has now been licensed to Oncocidia Ltd., London, UK.

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