Personalized functional profiling ofusing *ex- vivo* patient-derived spheroids points to out a the potential of an antiangiogenic treatment for in a patient with a metastatic atypical lung atypical carcinoid tumors

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Running head: Personalized functional profiling of *ex vivo* spheroids

Presentation:

Disclaimers:

Ethical approval

The patients participants provided tumor samples following informed consent. All the procedures performed were in accordance with the ethical standards of the institutional ethics

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Thank you for giving us an opportunity to review your manuscript. The technical expert comments have been structured into focus areas and recommended actions.
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The technical comments have been highlighted in grey.

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•A succinct title, which must be no longer than 175 characters (including spaces). ...

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committee and with the ~~Helsinki~~ Declaration of Helsinki ~~and the institutional ethics committee~~.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Luxembourg Institute of Health and the Ministry of Higher Education and Research ~~(MESR) funding,~~ under the Personalized Functional Profiling ~~(PFP)~~ P~~p~~rogram.

ABSTRACT:

Herein, we report on ~~This study outlines the case of~~ a patient with a ~~a~~ metastatic ~~lung~~ atypical lung carcinoid tumor who presented with ~~a~~ pleural effusion and progression of liver metastases after developing resistance to conventional chemotherapy~~treatments~~. Personalized functional profiling (PFP)~~, i.e. drug screening,~~ was performed using liver metastasis-derived~~in~~ *ex- vivo* spheroids ~~obtained from the patient's liver metastasis~~ to identify potential therapeutic options. ~~The~~ D~~d~~rug screening ~~results~~ identified~~revealed~~ cediranib, an antiangiogenic drug, as a hit drug ~~for this patient,~~ from a library of 66 Food and Drug Administration ~~(FDA)~~-approved ~~and~~ investigational drugs. The patient was administered a combination of bevacizumab and capecitabine on the ~~B~~basis ~~of~~ the PFP ~~results~~ and considering the ~~reported evidence of~~ clinical efficacy of this ~~bevacizumab and capecitabine~~ combination in treating gastro-intestinal neuroendocrine tumors~~, this combination was given to the patient~~. After 4~~Four~~ months ~~later~~, the pleural effusion and pleural carcinosis regressed and there was no evidence of progression of ~~the~~ liver metastasis ~~did not progress~~. The patient was stable for ~~experienced~~ 2 years after~~of a stable disease~~ receiving ~~under the~~ PFP-guided ~~personalized treatment~~therapy, but ultimately died after acquiring resistance to the treatment and disease progression.

KEYWORDS: Personalized functional profiling; drug screening; pharmacotyping; personalized medicine; precision medicine; spheroid~~s~~; neuroendocrine tumor~~s~~; lung carcinoid tumor; antiangiogenic therapy

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Commented [A14]: The guidelines for a case report mention the following:
Do not include an abstract.

I have not deleted the abstract. However, I have edited it and re-arranged the text to improve the flow. There were some components missing. I have highlighted these in the report shared with you.

Commented [A15]: Focus area: The Abstract should describe the relevance of the study

Recommended action: since the target journal does not require an Abstract for case reports, this comment may be irrelevant. However, if targeting another journal and maintaining the Abstract, please consider starting with a background sentence that succinctly explains the context and relevance of this case, before stating the aim. For example: "Metastatic atypical lung carcinoids are rare and have a poor prognosis due to (…). Herein we report…"

Commented [A16]: An abbreviation that appears in the abstract/document only once can be removed.

I have followed this rule throughout the document, without including a separate comment at each instance to avoid inserting too many comments.

Commented [A17]: Focus area: The Abstract should clearly describe the broad implications of the study

Recommended action: Please consider ending the Abstract with one sentence that highlights the clinical implications and/or learning value of this case.

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Introduction

Lung neuroendocrine tumors (NETs)~~neuroendocrine tumors~~ account~~are~~ for approximately 20% of all lung cancers. They ~~are~~ comprise~~d of~~ four subtypes~~:~~: typical carcinoids, atypical carcinoids, large-cell neuroendocrine carcinomas, and small cell lung carcinomas.[1] Carcinoids account for 1-2% ~~One to two percent~~ of lung cancers ~~is carcinoids~~.[2] Considerable advances have been made in ~~T~~the treatment of gastroenteropancreatic (GEP)-NETs ~~has achieved considerable advances~~ in recent~~the last~~ decades with the introduction of sunitinib, everolimus, somatostatin analogs, and peptide receptor radionuclide therapy ~~(PRRT)~~ (for somatostatin receptor-positive GEP-NETs) ~~in the therapeutic scheme~~.[3,4] The~~The~~ incidence of lung and ~~gastroenteropancreatic (~~GEP~~) neuroendocrine tumors (~~NETs~~)~~ has increased significantly ~~risen~~ over the last 40 years most ~~,~~ likely ~~due~~ owing to improved diagnosis.[3] However, everolimus remains ~~is still~~ the only ~~treatment approved by the~~ United States~~US~~ Food and Drug Administration (FDA)-approved drug treatment for ~~patients with~~ lung NETs, especially ~~in particular those suffering from~~ advanced, progressive, nonfunctional lung ~~pulmonary~~ NETs. Therefore, ~~thus the need for~~ more treatment options are needed~~in this indication~~.[1,3,5,6]

A~~One of the~~ major challenge~~s~~ in the management ~~managing~~ of cancer in general, and lung NETs in particular, is developing ~~identifying~~ personalized treatment ~~strategie~~s ~~that~~ allowing ~~increase~~ patients~~' chances~~ to benefit from anticancer therapy. Managing~~The scarcity of~~ this rare type of lung tumor requires ~~calls for~~ the involvement ~~contribution~~ of a multidisciplinary ~~experts~~team ~~in the management of the disease~~.[2] ~~The treatment of a m~~Metastatic pulmonary~~lung~~ carcinoid tumor treatment is palliative rather than ~~not expected to be~~ curative and focuses on ~~but~~ relieving ~~the~~ symptoms ~~caused by~~ associated with tumor growth ~~or~~and hormon~~al~~e replacement~~production~~.[2] ~~So far, C~~clinical trials on~~tackling~~ ~~the management~~

Commented [A20]: The journal guidelines mention that the text should be limited to 1,500 words or fewer (excluding the title page, references, figures, and tables). The original text was 1961 words. I have revised it to 1715 words.

Further reduction warrants extensive deletions that require your discretion. I would request you to go through the text and delete all details that you deem least important/inessential so that this limit can be met. I will be happy to check the revised text should you wish.

For your convenience, I have highlighted certain sections that could be deleted. Please review these carefully.

Commented [A21]: The guidelines mention the following: All references must be listed in the order in which they appear in the text. However, **you do NOT need to change the text style or order of individual reference elements to match Journal style**. We will automatically adjust reference formatting and verify data against PubMed-indexed citations after acceptance.

I have therefore not changed the format.

Commented [A22]: Focus area: Insufficient background information

Recommended action: before describing the treatment scenario, please consider adding a paragraph on the prognosis/mortality associated with lung carcinoids, as well as main challenges in treatment success. This information should be presented for carcinoids in general and for metastatic atypical carcinoids, which is the type in the present case.

Commented [A23]: "Due to" means caused by, and not because of. The rule of thumb when framing constructions with “due to” is as follows: if “due to” can be substituted with “caused by” without making the sentence sound improper, the expression is correct. If not, the expression needs to be revised to “because of” or “owing to.” For example, in “Due to the heavy traffic, we reached home very late,” the phrase beginning with “due to” is used to answer the question “why.” However, because “due to” is an adjectival expression, this sentence is incorrect and needs to be revised as “Because of the heavy traffic, we reached home very late.” However, in “The delay was due to the heavy traffic,” the phrase “due to the heavy traffic” tells us why the delay was ...

Commented [A24]: Focus area: Clarity / flow

Recommended action: for improved flow, please consider moving this sentence above, right after presenting the incidence of carcinoids. This is because the sentence seems to interrupt the flow of the text about treatments.

Commented [A25]: I have split large paragraphs into one or more paragraphs to aid in readability.

Commented [A26]: Focus area: Clarity of the Rationale

Recommended action: Since this is the main topic of the presented case, the rationale should be more thoroughly and clearly described. Please consider expanding this idea by clarifying the concept of personalized treatment, why personalized treatments are important/advantageous in general and specifically in oncology, and what are the main challenges for its development and use.

~~of~~managing advanced~~–~~stage pulmonary carcinoid tumors are~~remained~~ limited.~~,~~[7] ~~and~~ ~~p~~Personalized drug screenings ~~(on patient's tumor material, i.e.~~ functional tumor profiling or pharmacotyping~~)~~[8,9] of patient-derived material is important for making appropriate ~~are thus highly encouraged to issue~~ treatment recommendations.

Herein, we report the case of a patient with a metastatic lung NET who underwent personalized functional profiling (PFP) of his tumor and was treated based on ~~the~~ drug screening results.

Commented [A27]: Focus area: Insufficient background information / rationale clarity

Recommended action: Please consider explaining succinctly what personalized drug screening consists of, and complement citing some examples of its use in oncology.

Commented [A28]: I have moved this sentence to the end of the introduction as it is important to set context before mentioning what the report entails.

Case presentation

A 52-year-old man was diagnosed with an atypical carcinoid of the lung (pT2 pN1 ~~([~~1/25~~])~~ G2, ~~–~~10 mitoses/10 high~~-~~power fields ~~(HPF),~~ Ki-67 ~~=~~15%) in June 2009. R~~A r~~ight lower bilobectomy and systematic lymphadenectomy were ~~then~~ performed. In May 2012, the patient was admitted to Mannheim University Medical Center for~~to undergo a~~ undergoing a biopsy of the new lesions detected on~~found in~~ surveillance imaging and for suspected~~suspicious~~ ~~of~~ disseminated osteoplastic bone metastases. ~~The~~ ~~i~~Immunohistochemical staining ~~analysis~~ of bone sections~~material~~ showed strong and continuous expression of chromogranin A and weak ~~, but specifically~~ membrane-specific~~bound~~ co-expression of CD56. T~~he t~~umor cells stained~~were~~ negative for cytokeratin 7 ~~(CK-7)~~, cytokeratin 20 ~~(CK-20)~~, thyroid transcription factor ~~1 (TTF~~-1~~)~~, napsin A, prostate specific antigen~~PSA,~~ and prostate specific acid phosphatase expression ~~PSAP staining.~~ The m~~M~~itotic count was 4 per 10 high-power field ~~HPF~~s. T~~ and t~~he Ki-67 index ranged from~~between~~ 10% to~~and~~ 15%. ~~The~~A diagnosis of ~~a~~ disseminated hepatic and bone metastas~~ei~~s due to an ~~the clinically known lung~~ atypical carcinoid of the lung was made~~seen~~. The patient was treated with capecitabine and temozolomide from June 2012 to January

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Recommended action: the case is extremely thorough and well-described. However, the methodology of spheroid culture may not need to be so detailed for a case report. I suggest presenting that part as supplementary information.

2015.

Treatment ~~Therapy~~ was discontinued~~then interrupted~~ ~~due~~ owing to a ~~persistent~~ stable disease status. In November 2016, the disease progressed. ~~The~~ eExtensive tumor ~~bone marrow~~ infiltration of the bone marrow ~~tumor~~ precluded ~~a~~ peptide receptor radionuclide therapy ~~(PRRT)~~. In December 2016, the patient was retreated ~~exposed~~ with ~~to~~ capecitabine and temozolomide until ~~the~~ progression of ~~the~~ liver metastases in February 2017. Consequently, treatment ~~therapy~~ was switched to everolimus. ~~treatment. Evidence~~Evidence of pulmonary and hepatic progression~~progressive disease appeared~~ in January 2018 led~~leading~~ to discontinuation of everolimus.

The ethics committee of X was consulted before the patient was treated on an individual~~a single case~~ basis. The committee granted approval~~;, and~~ the patient provided ~~gave his~~ informed consent ~~prior to~~before the intervention. In March 2018, a re-biopsy of the liver showed a progressive NET (G3, Ki-67 =18%). A sample was ~~sent~~ transported to Ksilink (Strasbourg, France) for ~~personalized functional profiling (~~PFP~~)~~ ~~(, i.e.~~ drug screening of tumor-derived spheroids~~,~~ and identification of potential hit drug~~s)s~~. The ~~collected tumor~~ sample consisted of four~~4~~ core-needle biopsy specimen~~ies~~ ~~(corresponding to~~ 125.5 mg ~~in total; (a~~ minimum of two~~2~~ needle biopsy specimen~~ies~~ is generally ~~commonly~~ required for spheroid generation).

Biopsy specimens~~Briefly, the tumor biopsy~~ were~~was~~ mechanically and enzymatically dissociated ~~as follows~~: t~~T~~he ~~tumor was~~y were ~~washed~~ rinsed with cold DMEM/F12 medium supplemented with fetal bovine serum and antibiotics and minced into 1-3 mm^3 fragments using sterile forceps and a scalpel. Tumor fragments were ~~washed~~ rinsed and~~again then~~ digested in DMEM/F12 medium containing collagenase. The cells were seeded in complete StemPro~~TM~~ hESC SFM medium (Gibco) in ultra-low attachment ~~dishes,~~plates and incubated

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Recommended action: This whole paragraph is very similar to text of a previous publication. Please consider paraphrasing.

Commented [A31]: Please mention the name of the institution providing the approval.

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Recommended action: This whole paragraph is very similar to text of a previous publication. Please consider paraphrasing.

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Commented [A36]: For reagents used, the company that supplied the reagent and the catalogue number should be listed in parentheses; do not list the company location. Please check this at all relevant instances.

Commented [A37]: Please mention the concentrations and volumes used.

at 37°C in an atmosphere of 5% CO_2. ~~The c~~Cells were regularly observed~~inspected~~ under a microscope to check for spheroid formation. ~~The s~~Spheroids were passaged every few days via~~using a~~ mild enzymatic dissociation to avoid the accumulation~~ng~~ of dead cells in the center of the spheroid.

~~The s~~Spheroid culture was considered~~deemed~~ successful if ~~the~~ three-dimensional ~~3D~~ entities formed~~displayed~~ a typical ~~standard~~ rounded multicellular structure and ~~if they could outgrow~~ propagated in culture within a few days ~~in culture~~ and on~~propagate after~~ passaging. Figure 1(a) shows bright-field images of tumor~~patient~~-derived spheroids at different~~variable~~ passages and days after plating. Images were ~~,~~ generated from three~~3~~ different tumor specimens ~~(as~~ described above). PFP was performed on short-term cultured spheroids ~~in order~~ to deliver drug screening results ~~back~~ to the clinician within an acceptable timeframes~~.~~ ~~To do so, the s~~Spheroids were dissociated into single cells and small-~~-~~cell clusters and printed in an~~with the~~ alginate matrix of~~in~~ hanging drops onto 2-~~-~~mm diameter pillars in a 384-pillar plate (with a technical duplicate)~~,~~ using an~~the~~ ASFA Spotter ST (Medical & Bio Decision, Suwon, South Korea) [~~(~~Figure 1(b)~~)~~].

One day after ~~cell~~ printing, ~~the~~ cells were exposed to a library of 66 FDA-approved ~~and~~ investigational drugs in a 4~~four~~-fold 7~~and seven~~-point serial dilution ~~series~~ for ~~five~~5 days. Live cells were stained with calcein AM; ~~and~~ the plates were imaged using a high-throughput screening system. Cells~~The cells~~ were scanned at 4×~~x~~ magnification. C~~ and~~ ell~~their~~ viability was ~~assessed~~quantified as~~ by~~ the area of ~~the~~ calcein AM ~~live cell~~ staining and normalized to ~~the~~ that of DMSO-treated cells. For each drug, the half-maximal inhibitory concentration ~~(IC50)~~ and ~~D~~dose-~~R~~response ~~C~~curve ~~(DRC)~~ were generated~~;~~ ~~and~~ the ~~A~~area under the dose-response curve ~~DRC (AUC)~~ was calculated. To identify personalized drug candidates, we compared the drug sensitivity profiles of~~obtained from~~ the patient's tumor-derived spheroids

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Commented [A39]: Four core-needle biopsy specimens were taken. Should this be "four" rather than "three different tumor specimens"? Please check this.

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Recommended action: This whole paragraph is very similar to text of a previous publication. Please consider paraphrasing.

with the pharmacological landscape of tumor-derived spheroids from 11 other patients with cancer ~~patients' spheroids~~.

The patients' clinicopathological characteristics~~features of the patients~~ are summarized in Table 1 (P~~p~~atient 11 is the subject of this case report). A drug was considered ~~as~~ a "hit" ~~of interest for our patient~~ if the ~~AUC~~ z-score of the area under the dose-response curve was less than ~~-~~−1, indicating the inclusion of the patient's tumor-derived spheroids in the top 16% of the most sensitive spheroids to this drug. ~~Based on the d~~Drug sensitivity analysis~~,~~ revealed only cediranib ~~was selected~~ as a hit ~~drug~~ (z-score < ~~-~~−1) [~~(~~Figure 2(a)~~)~~]. O~~Interestingly, o~~ur patient's tumor was ~~the~~ most resistant to everolimus ~~([~~Figure 2(a)~~)~~], consistent~~which is in line~~ with ~~the~~ clinical evidence that ~~of~~ everolimus resistance manifested in this patient before ~~collecting the~~ tumor sampling~~sample used~~ for drug screening [~~(~~Figure 2(a)~~)~~]. The dose~~-~~response curves ~~of the patient's derived spheroids treated with cediranib and everolimus~~ showed~~shows~~ a dose-dependent cytotoxic effect of cediranib but not of ~~in these cells. In contrast, they were unresponsive to~~ everolimus ~~treatment~~ [~~(~~Figure 2(b)~~)~~]. Cediranib~~,~~ ~~is~~ a multi~~-~~kinase vascular endothelial growth factor (VEGF) receptor ~~(VEGFR)~~ inhibitor, has shown ~~that demonstrated~~ promising results in preclinical trials but failed ~~to meet its main goals~~ in ~~several~~ clinical studies.[10–12] ~~The~~ Next~~-G~~generation ~~S~~sequencing ~~assay~~ (Illumina TruSight~~TM~~ Tumor 170 gene panel ~~– Illumina®~~) of the liver metastasis~~metastatic~~ sample from March 2018 did not identify ~~reveal~~ any druggable targets. Therefore, PFP ~~and functional profiling~~ remained the only option for improving ~~the~~ the disease outcome.

On the basis~~Therefore, based~~ o~~n~~f ~~the~~ personalized drug screening ~~results~~ and ~~taking into account~~ the clinical efficacy~~activity~~ and safety profile of the combination of bevacizumab (a VEGF blocker) and capecitabine ~~combination~~ in gastro~~-~~intestinal NETs (~~-in the~~ BETTER trial),[13] the ~~i~~Institutional ~~t~~Tumor B~~b~~oard recommended treatment with bevacizumab and

Commented [A41]: If there are 11 patients in total with one being the subject of this case report, then this would be better phrased as "10 other patients with cancer." Please check this.

Please consider mentioning whether these 11 other patients also provided consent.

Commented [A42]: Focus area: Table 1 is not provided

Recommended action: please consider providing Table 1 and its corresponding legend.

capecitabine ~~and bevacizumab and~~. T~~t~~reatment was initiated in May 2018, after evidence of pleural effusion and progression of liver metastases (Figure 3). After 4~~Four~~ months ~~later~~, ~~the~~ pleural effusion and pleural carcinosis regressed and ~~the~~ liver metastas~~i~~es remained stable (Figure 3). The patient ~~maintained a~~was stable ~~disease~~ for 2~~during a two-year~~ years ~~period~~ after receiving ~~under the~~ PFP-guided ~~personalized treatment~~therapy. In June 2020, 25 months after the commencement of bevacizumab and capecitabine~~/bevacizumab~~ therapy~~started~~, ~~the disease progressed implying~~ acquired resistance and disease progression occurred ~~to this combination and~~. T ~~t~~he patient died 6 months later.

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Recommended action: This whole paragraph is very similar to text of a previous publication. Please consider paraphrasing.

Discussion

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Recommended action: Please check the minor comment below.

This ~~case~~ report highlights ~~gives~~ the importance of ~~a personalized functional profiling (~~PFP~~)~~ ~~approach~~ for personalized ~~therapy~~medicine, especially when no other treatment option is available~~expected to generate a promising clinical response~~. ~~Indeed, w~~With no druggable targets identified ~~, as revealed by~~on ~~the~~ genome~~genomic~~ sequencing~~.~~ and ~~with~~ the development ~~acquisition~~ of ~~secondary~~ drug resistance ~~to several therapies~~, the decision ~~of~~to pursue ~~pursuing~~ PFP ~~for drug recommendation, i.e.~~ (~~-~~drug screening of the patient's tumor-derived spheroids ~~generated from the patient biopsy,~~ and identification of potential drug hit ~~drug~~s) for drug recommendations allowed for ~~permitted~~ the identification ~~selection~~ of an antiangiogenic drug as a potential therapy and confirmation of ~~confirmed~~ the clinically observed resistance to previous treatment~~therapies~~. ~~Accordingly,~~ T~~t~~he patient benefited from ~~an additional two-year period from a~~ personalized therapy with stable disease status for an additional 2 years ~~treatment~~ based, in part, on our observation of the~~a~~ significant ~~activity~~efficacy of this class of drugs in an *ex -vivo* patient-derived spheroid model ~~from the patient's tumor~~.

AIn cancer, angiogenesis, i.e. the formation of new and abnormal blood vessels, is an important forfactor in tumor growth and metastasis.[14] The release of pro-angiogenic factors by cancer cells and the tumor microenvironment promotes stimulates the migration, and proliferation, and vessel formation inof endothelial cells and triggers vessel formation.[15] Apart from angiogenesis, oOther mechanisms contribute to account for tumor vascularization, especially in particular vessel co-option (, a process whereby tumorcancer cells incorporate and use preexisting vessels from the surrounding normal tissue instead of inducing new vessel growth) to proliferate and spread, and vascular mimicry (which is the acquisition by tumor cells of an endothelial-like phenotype by tumor cells, resulting inleading to vascular-like structures).[15,16] Previous studies have shown that NETs are highly vascularized, suggesting the possiblepotential efficacy of antiangiogenic drugs in treating these tumorsthis indication.[17,18] Based on this rationale, Tseveral clinical trials have been conducted to evaluate the efficacyactivity of antiangiogenic drugs in treating advanced NETs has been evaluated.[19–21] ThisThese investigations led to the FDA approval of sunitinib for treating in treating progressive, well-differentiated pancreatic NETs in for patients with locally advanced or metastatic disease.[22] NoAlthough no antiangiogenic drugs have been granted FDA approval for lung NETs to date. However, of pulmonary origin yet, several clinical trials have already demonstratedshown the efficacy of antiangiogenic drugs, ir activity in this indication, as illustrated by the results of studies investigatingincluding surufatinib,[20] axitinib,[23] pazopanib,[24] and bevacizumab,[25] for treating NETs. in NET. Nevertheless, there is no consensus on general treatment recommendations can be given at this stage regarding using antiangiogenic drugs for treating lungin NETs of pulmonary origin. Resistance to Evasion of anti-angiogenic therapy after an initial response phase has been reported in several metastatic cancers.[26–28] In thouris case report, the patient manifestedacquired resistance to the combination of capecitabine and /bevacizumab

Commented [A45]: This is a common medical term requiring no further explanation and thus has been deleted.

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Recommended action: This sentence is very similar to that of a previous publication. Please consider paraphrasing.

~~combination~~ after 2~~two~~ years of treatment, suggesting ~~. This acquired resistance suggests~~ the activation of adaptive and compensatory mechanisms~~,~~ (~~-~~e.g., up~~-~~regulation of pro~~-~~angiogenic factors other than VEGF [a (~~target~~ of~~ed by~~ bevacizumab]~~)~~, vascular co-option, and vascular mimicry).[15,16] The cancer stem cell population may ~~also~~ be involved in ~~the secondary~~ drug resistance and ~~tumor~~ relapse~~, as previously shown~~.[29,30] Unfortunately, ~~the degradation~~ deterioration of the patient's health precluded additional ~~condition was not anymore compatible with a new~~ tumor sampling ~~procedure, and thus a~~preventing follow-up PFP ~~could not be envisaged~~.

Among ~~the~~ three-dimensional ~~(3D)~~ models used in preclinical research, patient-derived xenograft~~s (PDX)~~ models have contributed significantly ~~contributed~~ to ~~the~~advancements in ~~advancing~~ precision medicine and are widely~~still largely~~ used in biomedical research.[31] ~~Nevertheless~~However, some limitations of these three-dimensional~~3D~~ preclinical models exist ~~are to be mentioned; ~~: the~~The~~ potential ~~possible~~ contribution ~~to the observed drug response~~ of factors inherent to the ~~animal~~ model itself to the observed drug response;~~,~~[32] the lengthy ~~procedure~~ (6~~ to~~ 8 month~~s~~) procedure required for ~~the generation of~~generating patient-derived xenograft ~~these PDX~~ models, which may not be compatible with the rapid progression of the disease;[33,34] and ~~and~~ the limited number of protocols that can be used~~tested~~.[31]

Contrary~~Contrarily~~ to patient-derived xenograft ~~a PDX~~ model~~s~~, our PFP approach (which exploited ~~exploiting~~ the predictive potential of an *ex* ~~-~~*vivo* patient-derived spheroid model)~~s~~[35,36] ~~could~~ provide~~d~~ treatment recommendation~~s~~ within ~~i~~2~~n less than two~~ months~~__~~, a clinically ~~still~~ acceptable timeframe for treatment decision-making ~~in~~ for patients with cancer ~~patients~~. Importantly, our ~~cell~~ culture conditions support~~ed~~ the growth and propagation of stem cells, suggesting that at least a fraction of the spheroid-forming cells are ~~forming the~~

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Recommended action: please consider explaining the reasons for this limitation (or, in alternative, delete the sentence since the incompatibility between treatment development vs disease progression is likely the main challenge).

Commented [A49]: This sentence is rather vague. Please clarify which protocols are being referred here.

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~~spheroids is~~ cancer cells with stem ~~-~~cell-like features that may contribute to ~~tumor~~ relapse ~~in the clinic~~. ~~In addition~~Moreover, our animal-free approach is easily applicable in a hospital environment. ~~Such a~~ Ppersonalized ~~strategy is~~strategies are needed, ~~highly required~~ especially for aggressive and ~~in~~ multidrug-~~-~~resistant tumors that are ~~,~~ poorly ~~studied~~understood, ~~aggressive tumors,~~ as is the case ~~of~~for ~~this~~ a metastatic ~~lung~~ atypical lung carcinoid tumor.

Commented [A51]: Please consider adding the following: The primary "take-away" lessons from this case report (without references) in a one paragraph conclusion.

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Acknowledgments

We thank the patients who participated ~~for their participation~~ in this study and the ~~physicians~~clinicians for their ~~collaboration~~cooperation.

References

Commented [A52]: The journal guidelines mention the following about reference format:
You do NOT need to change the text style or order of individual reference elements to match Journal style. We will automatically adjust reference formatting and verify data against PubMed-indexed citations after acceptance. Any necessary edits will be made at copyediting so that references conform to the sample styles (as shown below) at publication.
Journal article with one, two, or three authors
1. Dolan ME, Pegg AE: O6-Benzylguanine and its role in chemotherapy. Clin Cancer Res 8:837-847, 1997
Journal article with more than three authors
2. Knox S, Hoppe RT, Maloney D, et al: Treatment of cutaneous T-cell lymphoma with chimeric anti-CD4 monoclonal antibody. Blood 87:893-899, 1996
Journal article in press (manuscript has been accepted for publication)
3. Scadden DT, Schenkein DP, Bernstein Z, et al: Combined immunotoxin and chemotherapy for AIDS-related non-Hodgkin's lymphoma. Cancer (in press)
Supplement
4. Brusamolino E, Orlandi E, Morra E, et al: Analysis of long- term results and prognostic factors among 138 patients with advanced Hodgkin's disease treated with the alternating MOPP/ABVD chemotherapy. Ann Oncol 5:S53-S57, 1994 (suppl 2)
Book with a single author
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Table

Table 1.

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Cite tables in the order in which they appear in the text using Arabic numerals. The legend should include any pertinent notes and must include definitions of all abbreviations and acronyms used in the table.
Please place tables at the end of the manuscript.

Figure 1. Representation of the micropillar-based drug screening workflow for personalized functional profiling.

Figure 2. Drug sensitivity profiles of ex-vivo patient-derived spheroids.

Figure 3. Chest and abdominal/pelvic computed tomography ~~(CT)~~ scans during capecitabine/bevacizumab treatment.

Source: Personalized functional profiling using ex-vivo patient-derived spheroids points out the potential of an antiangiogenic treatment in a patient with a metastatic lung atypical carcinoid by Hichul Kim, Victoria El-Khoury, Nadine Schulte et al., used under CC-BY

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All Kaplan-Meier plots must include risk tables.

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Commented [A58]: In the above figure, we have revised "screen" to "screening".