

## The cell of cancer origin provides the most reliable roadmap to its diagnosis, prognosis (biology) and therapy

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### ABSTRACT

Cancers arise from single transformed cells from virtually every organ of the body, divide in a relatively uncontrolled manner, and metastasize widely. A search for a “magic bullet” to precisely diagnose, characterize, and ultimately treat cancer has largely failed because cancer cells do not differ significantly from their organ-specific cells of origin. Instead of searching for genomic, epigenetic, transcriptional, and translational differences between cancers and their cells of origin, we should paradoxically focus on what cancer cells have in common with their untransformed cells of origin. This redirected search will lead to improved diagnostic and therapeutic strategies where therapeutic index considerations and drug-limiting toxicities can largely be circumvented. We cite three cancer examples that illustrate this paradigm-shifting strategy: pseudomyxoma peritonei (PP), metastasis of unknown origin (cancers of unknown primary) (MUO), and cancers that arise from potentially dispensable organs (CAD). In each of these examples, the cell of cancer origin still provides the most reliable road map to its diagnosis, prognosis (biology), and therapy.

### Introduction

Cancers arise from singly transformed cells from virtually every organ of the body, divide in a relatively uncontrolled manner, and metastasize widely [1]. Cancers represent common diseases whose incidence is thought to increase exponentially with age [2,3]. Although cancers have many different causes, a common theme in cancer is that they represent a disease involving our own cells in contrast to infectious diseases, which are caused by foreign organisms [4,5]. As a result, a search for a “magic bullet” to precisely diagnose, characterize, and ultimately treat cancer has largely proven futile because cancer cells do not differ significantly from their organ-specific cell of origin [6]. The multi-hit theory of carcinogenesis has been cited to help elucidate concepts such as cancer latency, the time interval between cancer initiation and tumor detection, and the relationship between cancer and aging, where multi-hit accumulation is needed for complete cancer emergence [7,8]. In classic carcinogenesis theory, initiators and promoters act on cells in several steps or “hits”, setting in motion the process of transformation, tumorigenicity, invasion and metastasis [9,10]. Classic carcinogenesis theory assumes that this accumulation of multiple hits, which occurs over many years, is both necessary and sufficient for

full cancer emergence [11]. “Hits” are considered to be genetic and defined by mutations, rearrangements, or amplifications in oncogenes or deletions or mutations in tumor suppressor genes [12]. However, the number of “hits” is estimated to be finite and usually in the range of 3–7, a small number compared to the 10,000–100,000 normal genes expressed in both cancer and their untransformed cells of origin [13]. Furthermore, not every spontaneous cancer is due to multiple hits. The ABL/BCR translocation within the Philadelphia chromosome is the single hit responsible for chronic myelogenous leukemia (CML), and spontaneous retinoblastoma is also the result of a single RB mutational hit [14]. In the setting of germline or inherited cancer, where a defective tumor suppressor gene or oncogene is inherited, the number of “hits” may also be minimal. In many germline recessive mutations that trigger cancer, a single mutated allele invariably causes a second allelic derangement in a dominant manner, setting in motion cancer transformation. Additional hits are not required. The relatively high penetrance of germline BRCA1/BRCA2, P53 (in the Li-Fraumeni Syndrome), RB, APC, and RET suggest that, for their associated cancers, multiple hits are not required [14–22]. In the settings of these “limited-hit cancers”, the differences between the cancers and their cells of origin would be expected to be de minimis.

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Furthermore, most scientists believe that cancers contain a significant stem/progenitor cell compartment [23–25]. The evidence for this belief is strong and multifaceted. For one, only the stem cell or progenitor cell subpopulation of a cancer is capable of self-renewal and multipotency. The proliferating subpopulation of a cancer is susceptible to radiation therapy and chemotherapy among other antiproliferative strategies, but stem cells and progenitor cells resist such antiproliferative strategies. Stem and progenitor cells express different stem cell-associated genes and pathways, and have biomarkers of stemness that distinguish them from other tumor subpopulations. Stem and progenitor cells are thought to be largely responsible for tumor relapses and recurrences [23–25]. Although tumor stem/progenitor cells represent a tumor compartment capable of self-renewal and multipotency, accounting for cancer relapses and recurrences, tumor stem/progenitor cells are thought to be most likely derived from transformed normal stem cells contained within the tissue or organ of cancer origin. Although cancers are common diseases, such that The American Cancer Society estimates, by the age of 80, 37.7% of females and 39.3% of males (over one in three) will have developed an invasive cancer [2], the overwhelming majority of both spontaneous as well as inherited cancers arise from only a single focus and are thought to be monoclonal proliferations arising from a single transformed cell [5,11]. Usually, only 1 out of  $10^9$ – $10^{12}$  cells in any given organ ever transform. The reasons for this are not completely clear, but one recent hypothesis is that only cells at a critical window of differentiation are primed for transformation [5,26].

It is important to emphasize that the rare cell that does transform at the primary site is a cell with the original genetic and epigenetic signature of the primary site, a cell, if you will, with an IP address. In any case, it would be expected that cancers derived from this transformed cell would reflect their cellular origin. Moreover, from that perspective, the cell of cancer origin should provide the most reliable roadmap to its diagnosis, prognosis (biology), and therapy.

## The observation

Despite the paucity of differences between the cancer cell and its cell of origin and notwithstanding the abundance of their similarities, we have become obsessed with looking for a “magic bullet” that will selectively target the cancer cell and spare normal cells of the host. Our obsession with this magic bullet comes from the classic studies of Paul Ehrlich in infectious disease [4,6], which have been predicated on the significant differences between prokaryotes and eukaryotes. Cancer cells, compared to normal cells, have much fewer differences. The “magic bullet” in cancer is essentially based on three classes of differences [4,6]: 1) differences in rates of cell division between cancer cells and normal cells that can be exploited; 2) differences in oncogene or tumor suppressor gene proteins, which predominantly affect the cell cycle and that can be blocked, and 3) differences in tumor-associated or tumor-specific antigens that can be immunologically manipulated either through immune enhancement or reversing immune suppression with checkpoint inhibitors. However, each of these approaches has been limited by dose-limiting toxicities and therapeutic index restrictions. Many normal cells divide as rapidly as cancer cells, and cell division alone has certainly not provided a unique target. The protein products of mutated, rearranged or amplified oncogenes or mutated or deleted tumor suppressor genes can not usually be blocked specifically or efficiently [27,28]. Although there may be tumor-associated antigens, there are very few tumor-specific antigens. The vast majority of tumor antigens, in fact, are not unique to tumor cells but rather shared with normal cells. Even in the setting of microsatellite instability and mismatch repair deficiency, where many tumor-specific antigens are generated, many of these antigens do not necessarily turn out to be significant immunogens. So, for the vast majority of cancers, the immune system can not be exploited to the point of being a “magic bullet” [4,6].

**Table 1**

Discriminatory Biomarkers of Pseudomyxoma Peritonei (PP), Primary Appendiceal Mucinous Neoplasms, Primary Ovarian Mucinous Neoplasms, and Primary Peritoneal Mesotheliomas\*

	Pseudomyxoma Peritonei (PP)	Primary Appendiceal Mucinous Neoplasms	Primary Ovarian Mucinous Neoplasms	Primary Peritoneal Mesotheliomas
CK7	–	–	+	–
CK20	+	+	–	–
SATB	+	+	–	–
PAXS	–	–	+	–
Leptin	+	+	–	–
mTOR	+	+	–	–
CA125	–	–	+	–
K-RAS	+/-	+/-	+/-	+/-
MUC-1	–	–	+	–
MUC-5AC	+	+	+	–
MUC-2	+	+	–	–
Calretinin	–	–	–	+
WT-1	–	–	–	+
Protein				

\* Biomarkers represent positive (+) or negative (–) immunoreactivity in a significant plurality of cases but not all. Note the strong correlation between the pattern of biomarker expression in PP and primary appendiceal mucinous neoplasms, suggesting the site of origin.

## The hypothesis

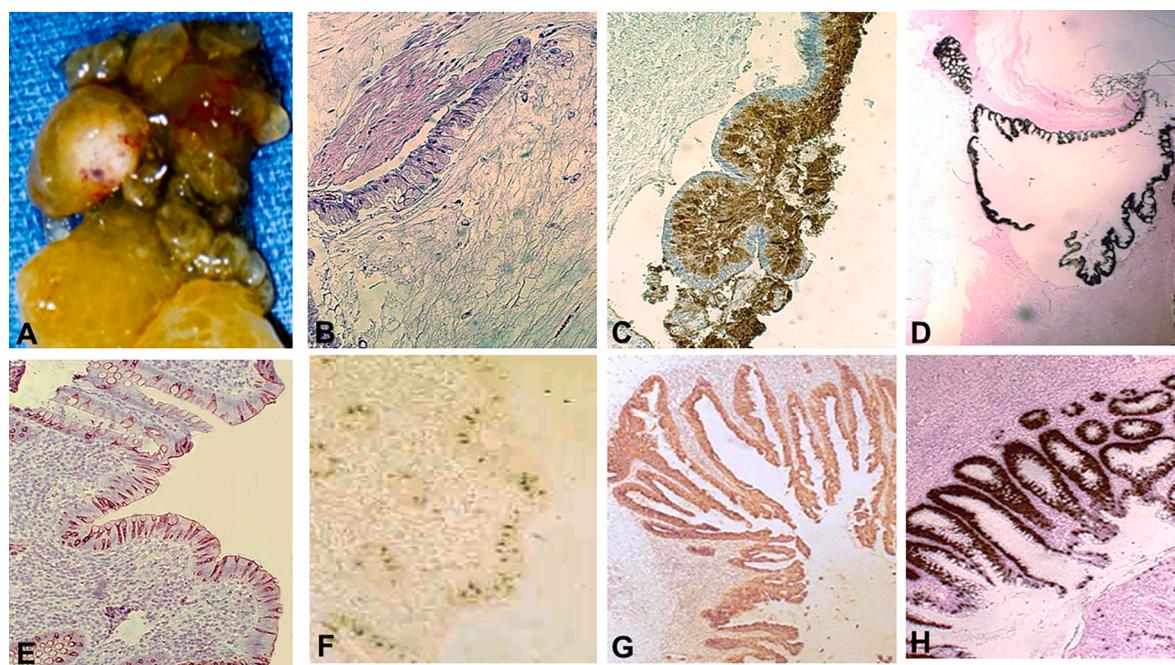
The bottom line is that cancers have much more in common with their cell of origin than they have differences. Instead of searching for genomic, epigenetic, transcriptional, and translational differences between cancers and their cells of origin, we should focus paradoxically on what cancer cells have in common with their untransformed cells of origin. To date, what cancers have in common with their cell of origin has been tacitly investigated to only a limited degree. Why not investigate these similarities to a much greater degree to better diagnose, understand the prognosis (biology), and ultimately treat the disease? This paradigm-shifting hypothesis could lead to improved diagnostic, prognostic, and therapeutic strategies where therapeutic index considerations and drug-limiting toxicities may largely be circumvented.

## Evidence for the hypothesis

We cite three cancer examples that illustrate this paradigm-shifting strategy: pseudomyxoma peritonei (PP) [29–55], metastasis of unknown origin (MUO) or cancers of unknown primary (CUP) [56–74] and cancers that arise from potentially dispensable organs (CAD) [75].

### Pseudomyxoma peritonei (PP)

According to O’Connell et al. [29], “Pseudomyxoma peritonei (PP), a syndrome first described by Karl F. Rokitansky in 1842 [45,52], is an enigmatic, often fatal intra-abdominal disease characterized by dissecting gelatinous ascites and multifocal peritoneal epithelial implants secreting copious globules of extracellular mucin. Although past interest in the syndrome has focused on the questions of the site of origin (appendix versus ovary), mechanisms of peritoneal spread (multicentricity, redistribution phenomenon, or metastasis), and the degree of malignant transformation present (adenoma, borderline tumor, or carcinoma), another important question is the mechanism behind the accumulation of extracellular mucin, the real cause of the disease’s morbidity and mortality irrespective of the site of origin, mechanism of peritoneal spread, or transformed status of its epithelium”. In the past several decades, the source of PP was considered to be either a primary mucinous tumor of the appendix [30,55], a primary mucinous tumor of the ovary [54], or a primary peritoneal mesothelioma with mucinous (myxomatous) degeneration [53]. A number of case studies, however, noted the



**Fig. 1.** Cases of pseudomyxoma peritonei. Pseudomyxoma peritonei is an enigmatic disease characterized by copious accumulations of mucinous deposits throughout the abdomen. The so-called “jelly belly” (A). These deposits are largely composed of extracellular mucin secreted by neoplastic epithelium (B). Previous studies [29,30,55] have characterized the mucin as predominantly MUC-2 by both immunocytochemical studies (C) as well as in situ hybridization studies (D). Greater than 95% of the cases of pseudomyxoma peritonei arise from neoplasms of the appendix. The cell of origin is thought to be the goblet cell or the goblet cell stem / progenitor cell which also constitutively expressed MUC-2 by both immunocytochemistry (E) as well as in situ hybridization studies (F) at the same levels per cell as the cases of pseudomyxoma peritonei (C, D). Transformation of the goblet cells or the goblet cell stem / progenitor cells results in a neoplastic proliferation of goblet cells resulting in a mucinous tumor of the appendix which expresses MUC-2 by immunocytochemical (G) as well as in situ hybridization studies (H). (Portions of this composite figure are reproduced with permission from Fig. 1,2 and 4 of [29] O’Connell JT, Tomlinson JS, Roberts AA, McGonigle KF, Barsky SH. Pseudomyxoma peritonei is a disease of MUC2-expressing goblet cells. Am J Pathol 2002;161:551–64. [https://doi.org/10.1016/S0002-9440\(10\)64211-3](https://doi.org/10.1016/S0002-9440(10)64211-3) by Agreement between Dr. Sanford Barsky and Elsevier via the Copyright Clearance Center).

stronger association of PP with mucinous tumors of the appendix including mucoceles [50,51].

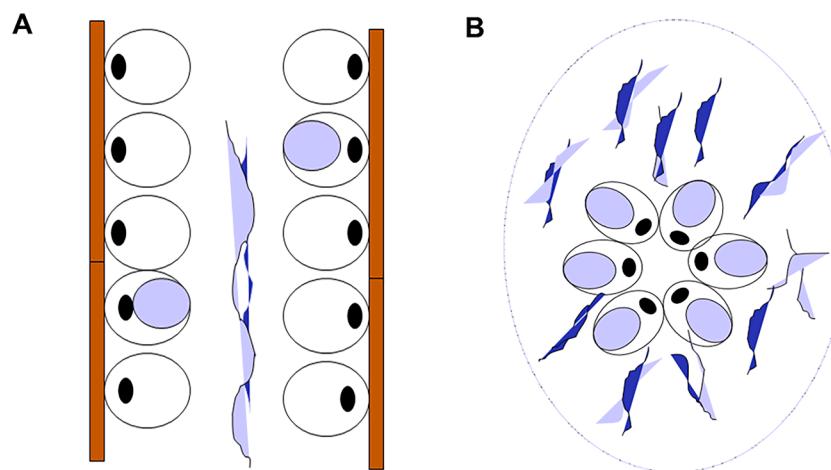
Because of the histological similarities between appendiceal mucinous neoplasms and ovarian mucinous neoplasms on routine light microscopy using standard hematoxylin and eosin staining, immunocytochemical studies exploiting known tumor biomarkers have been employed over the past two decades to help resolve the issue of whether PP takes origin from within the appendix or ovary. Based on a comparison of appendiceal epithelial, peritoneal mesothelial, and ovarian epithelial biomarkers and biomarkers expressed by PP, it was concluded that the overwhelming majority of PP cases (greater than 95%) emanate from mucinous neoplasms of the appendix [29,30,55] and not the ovary nor peritoneal mesothelium (Table 1). These findings are significant because they demonstrate the importance of determining and utilizing the cell of origin of a given cancer as the most reliable roadmap to its diagnosis, biology (prognosis), and therapy. The most useful biomarkers (both positive and negative) used in distinguishing mucinous neoplasms of the appendix from mucinous neoplasms of the ovary and demonstrating that PP nearly always arises from appendiceal mucinous neoplasms included CK7, CK20, SATB2, PAX8, CDX2, leptin, mTOR, CA125, K-RAS, MUC-1, MUC-5AC and MUC-2. Distinguishing primary peritoneal mesotheliomas with mucinous (myxomatous) degeneration from these other two entities utilized calretinin and WT-1 protein, which were strongly positive in the former but completely negative in the latter [53].

Meagher et al. [31] found that presence of both CK7 and SATB2 was both sensitive and specific for differentiating between primary ovarian mucinous neoplasms and primary colorectal / appendiceal mucinous neoplasms. They analyzed 155 primary ovarian mucinous neoplasms and 230 lower GI neoplasms (of which 107 were appendiceal mucinous neoplasms). They investigated CK7, CK20, CDX2, PAX8 and SATB2. CK7 was positive in 29.0% of appendiceal tumors but 97.4% of ovarian

tumors and SATB2 was positive in 87.9% of appendiceal tumors but only 12.9% of ovarian tumors. CK20, PAX8 and CDX2 were less discriminatory. Aldaoud et al. [32] similarly demonstrated the importance of CK7 and SATB2 in differentiating between ovarian mucinous neoplasms and those of colonic / appendiceal mucinous origin but, in addition, found PAX8 and CK20 to be discriminatory. They performed immunostaining on 50 ovarian mucinous neoplasms and 9 appendiceal mucinous neoplasms; SATB2 was expressed in 2.0% of ovarian tumors but 77.8% of appendiceal tumors; CK7 was expressed in 78.0% of ovarian tumors but only 33.3% of appendiceal tumors; CK20 was expressed in 24.0% of ovarian tumors but 88.9% of appendiceal tumors; PAX8 was positive in 32% of ovarian tumors but negative in all appendiceal tumors. In another study, Guo et al. [34] found that 33 of 35 PP cases were positive for MUC-2 and CK20, but negative for MUC-1, CA125, CK7, ER, and PR. Their results confirmed that appendiceal mucinous neoplasms were the source of PP.

In another study, Szych et al. [38] analyzed CK7 and CK20 expression in addition to K-RAS in 17 cases of PP with synchronous appendiceal and ovarian mucinous neoplasms. They concluded that PP was more likely derived from appendiceal mucinous neoplasms (CK20+, CK7-) than from primary ovarian mucinous tumors (CK7+, CK20-). The results of the K-ras mutational analysis also strongly supported the conclusion that the mucinous tumors involving the appendix and ovaries in women with PP were clonal and derived from one site, ie. the appendix. Thus, PP originates in the appendix and then spreads to secondarily involve other sites, including the peritoneum and even the ovary.

Using other biomarkers, Chang et al. [33] found that leptin, mTOR, MUC-2 and MUC-5AC were expressed in appendiceal mucinous neoplasms and in cases of PP and hypothesized that leptin may collaborate with both MUC-2 and MUC-5AC.



**Fig. 2.** Schematic of pseudomyxoma peritonei. Schematic depicts the production of mucin by the goblet cells of the appendix (A). Pseudomyxoma peritonei represents a neoplastic clonal proliferation of these mucin-producing goblet cells (B). Unlike in the appendix where the mucin is secreted and washed away in the gastrointestinal lumen (A), in the abdomen, it has nowhere to go and accumulates as the “jelly belly” (B). (Portions of this composite figure are reproduced with permission from Figure 8 of [29] O’Connell JT, Tomlinson JS, Roberts AA, McGonigle KF, Barsky SH. Pseudomyxoma peritonei is a disease of MUC2-expressing goblet cells. Am J Pathol 2002;161:551–64. [https://doi.org/10.1016/S0002-9440\(10\)64211-3](https://doi.org/10.1016/S0002-9440(10)64211-3) by Agreement between Dr. Sanford Barsky and Elsevier via the Copyright Clearance Center).

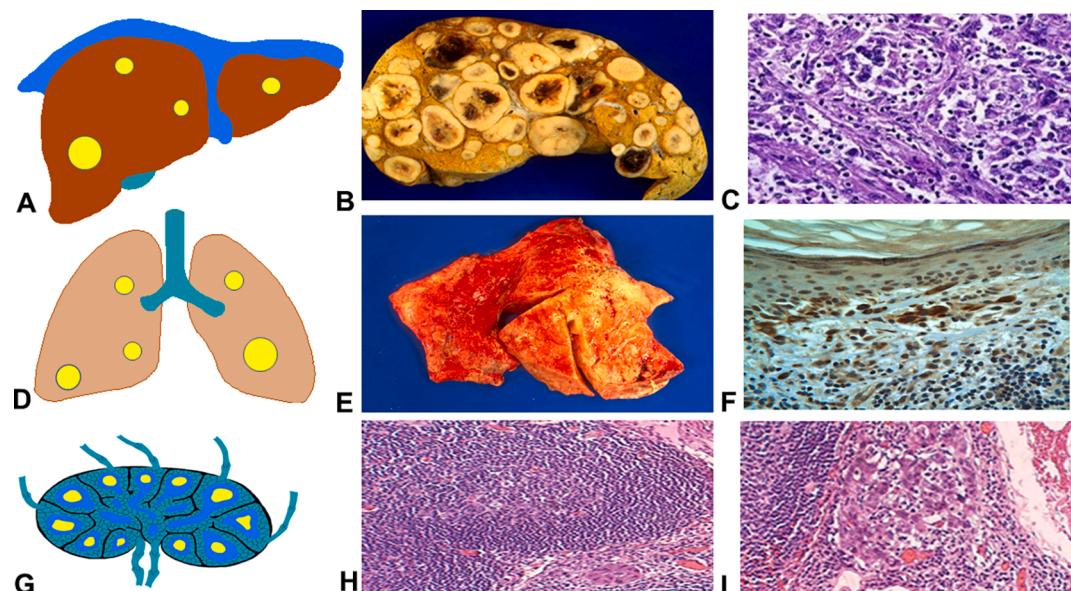
Of all the biomarkers useful in determining the cell of origin of PP, the most important biomarker has been MUC-2 (Fig. 1) [29,30,55]. MUC-2 is one of a handful of mucin genes cloned over the past two decades and has, along with MUC-5AC, produces a protein with the physicochemical property of being gel-forming [29,55]. MUC-2 and MUC-5AC are both expressed in PP and in the goblet cells of the appendix, but MUC-5AC expression is less specific, being expressed in mucinous ovarian tumors as well [29]. Unlike the other biomarkers, MUC-2 not only is a diagnostic marker of PP, mucinous tumors of the appendix and normal appendiceal goblet cells, but also its over-expression may represent the actual cause of PP's morbidity and mortality (Fig. 2) [29,55]. MUC-2 expression, indeed, is the significant factor leading to excess deposition of extracellular mucin (mucin: cell ratio greater than 10:1) [29]. MUC-2 therefore represents a potential molecular target. Interestingly, Semino-Mora et al. [35] found that gram-negative organisms can upregulate MUC-2 expression and, in fact, detected enteric bacteria in cases of PP. O’Connell et al. [29] similarly found that *Pseudomonas aeruginosa* lipopolysaccharide could upregulate MUC-2 expression but that the effect could be reversed with genistein. These findings suggested that MUC-2 could be pharmacologically regulated and that PP could be potentially treated in this manner.

Not only do biomarkers present within the PP cell of origin provide the most reliable roadmap to its diagnosis, but also to its biology (prognosis). PP, appendiceal mucinous neoplasms, and mucinous neoplasms of the ovary, stage for stage, for example, have a different prognosis.

Patients with localized mucinous tumors of the appendix and patients with PP (from a mucinous neoplasm of the appendix) have, stage for stage, a better prognosis than patients with mucinous ovarian neoplasms. In a study by Choudry and Pai [44], the authors divided appendiceal tumors into low, intermediate and high grade and found the 5 year overall survival to be approximately 86–91%, 61–63% and 23–32%, respectively. The presence of significant acellular mucin was an indicator of a more favorable prognosis. In a similar study, Reghunathan et al. [41] examined post-treatment mucinous appendiceal tumors with peritoneal dissemination and their histologic predictors, and found that the peritoneal histology helps predict progression-free survival (PFS). For example, with patients exhibiting high-grade peritoneal histology, the authors noted a median PFS of 16.8 months, while those with low grade histology had a median PFS of 34.4 months. In another study, Yan et al. [40] divided PP into two subsets of differing grade: low- and high-grade mucinous carcinoma peritonei. They studied additional biomarkers potentially related to tumor development, differentiation, relapse and metastasis, as well as clinical prognosis, which included expression levels / patterns of Ki-67 and P53. Multivariate analysis, however, identified histologic grade as the only significant prognostic factor.

In contrast, ovarian mucinous tumors, stage for stage, have a worse prognosis. Schiavone et al. [39] examined a SEER registry for women diagnosed between 1988 and 2007. They identified a total of 40,571 women with ovarian cancer out of which 4811 were mucinous carcinomas; 5-year survival was found stratified by stage: 1A (87.0% with n = 1915), 1B (75.1% with n = 81), 1C (78.3% with n = 546), II (54.7% with n = 323), III (25.7% with n = 1006), IV (10.2% with n = 842). In their 2018 study, Ricci et al. [43] elucidated that advanced mucinous tumors, found in women, had a worse prognosis compared to any other histological type. Their results suggest a 12-month median overall survival (OS) for women with mucinous tumors and a 37-month median OS for women with nonmucinous malignancies. Additionally, advanced stages (III/IV) were found to have a much worse prognosis compared to high-grade serous ovarian carcinoma (median OS of 14.6 vs. 40.6). Mucinous ovarian carcinomas were found to have the worst median OS for any histological subtype (OS of 47.7 months for serous carcinomas, 36.6 months for clear-cell carcinomas, but only 15.4 months for mucinous). In their study, the survival of women with stage I ovarian cancer with mucinous subtype was quite similar to those with serous neoplasms. However, in higher stages, 5-year survival was found to be much worse in mucinous tumors compared to serous (5-year survival of 10.2% vs. 20.3%, respectively). In another study by Brown and Fru-movitz [46], the authors found that prognosis of primary mucinous ovarian carcinomas were overall better compared to their serous counterparts. However, they found that advanced stage mucinous ovarian cancers had a significantly worse prognosis. This was supported by median PFS data, which showed only 5.7 months survival in patients with advanced stage mucinous ovarian cancer compared to 14.1 month survival for those with non-mucinous subtypes. OS was also worse, with the median being 12 months for advanced stage mucinous ovarian carcinomas compared with non-mucinous ovarian cancer (36.7 months). Mucinous ovarian carcinoma patients at advanced stages usually die of their disease.

In a study of therapy for PP and mucinous appendiceal tumors, Ramaswamy [48] found that the median survival rate and median progression-free survival rate was 196 months (16.3 years) and 98 months (8.2 years), respectively, with a 10-year survival rate of 63 % and 15-year survival rate of 59%. Better patient outcomes were seen in those with low-grade tumors than those with high-grade tumors ( $p < 0.001$ ). By determining disease severity using a peritoneal cancer index (PCI), this study found that patients with less extensive disease had improved outcomes compared to those with more extensive disease ( $p = 0.013$ ). Combining the modalities of cytoreductive surgery and hyperthermic intraperitoneal chemotherapy for treatment resulted in 62.5%–100% 5-year survival for low grade disease and 0%–65% 5-year survival for high grade disease. In another study of therapy, Carr et al. [47] analyzed one institution’s cases of PP surgery, and out of 274 cases, PP



**Fig. 3.** Cases of metastases of unknown origin (MUO). MUO can be divided into cases with visceral metastases (A,B,D,E) and lymph node metastases (G,H). The two most common sites for visceral metastases in MUO are the liver (A,B) and the lung (D,E). Possible explanations for MUO include primary cancer of very small size that avoids detection by routine imaging studies, primary cancer which undergoes spontaneous regression with scarring, eg. testicular germ cell neoplasia (C), primary cancer which undergoes regression by immune mechanisms, eg. regressed halo melanoma (F) and primary cancer which arises within an ectopic location, eg., ectopic breast ducts within the axilla (I).

was low grade in 78% of patients and high-grade in 22%. The authors further suggested an association with overall survival. The overall 5-year survival rate of low-grade and high grade were 63% and 23%, respectively. For those who underwent complete surgical cytoreduction, 5-year overall survival rates increased to 84% for low-grade and 48% for high-grade, with median survivals of 7.7 years and 2.8 years, respectively.

In yet another study of therapy, Wang et al. [42] analyzed 39 patients with PP who received treatment between 2002 and 2011. Every patient underwent cytoreductive surgery, and 7 patients also utilized hyperthermic intraperitoneal chemotherapy. Out of all 39 patients, only 25 cases of PP recurred, with median OS of 37 months, median recurrence time of 4 months, 5-year survival of 89.0%, and 10-year survival of 35.0%. For PP patients, the combination of surgery and intraperitoneal chemotherapy effectively extended recurrence time post-operation and improved quality of life. In another study of therapy of PP, Shaib et al. [36] found that for localized mucinous appendiceal tumor, simple appendectomy could suffice, but in order to clear tumor margins for more locally advanced diseases, performing a right hemicolectomy would be necessary. 5-FU-based adjuvant chemotherapy was not recommended for well-differentiated tumors, but rather for cases of tumors with poorer differentiation (signet ring histology) and signs of perforation or lymph node involvement. For mucinous appendiceal tumors with peritoneal involvement, cytoreductive surgery followed by hyperthermic intraperitoneal chemotherapy was recommended. Preoperative chemotherapy was also considered for high-grade but not low grade appendiceal adenocarcinomas. After an initial response, cytoreductive surgery was followed by hyperthermic intraperitoneal chemotherapy. For tumors containing K-ras mutations or COX-2 / K-RAS mutations, 5-FU / oxaliplatin or cetuximab / panitumumab was used, respectively. Overall response rate was 38% with 1-yr survival 84% and 2-year 61%. The authors also found that presence of mucinous ascites was indicative of a worse prognosis. 3, 5, 7, and 10 year survival for lower grade mucinous neoplasms of appendiceal origin with peritoneal spread was 100%, 86%, 60%, and 45%, respectively. Combining cytoreductive surgical therapy with hyperthermic intraperitoneal chemotherapy resulted in a higher 5-year survival rate, with that rate lowering for patients who only received cytoreductive surgical therapy or had

incomplete cytoreduction. The median OS for patients with PP who were treated with cytoreductive surgical therapy with or without hyperthermic intraperitoneal chemotherapy was 16.3 years, with a median PFS of 8.2 years. Incomplete cytoreduction was associated with poorer outcome. In another study of PP, Amini et al. [49] reported 16.3 years of median survival, 8.2 years of median PFS, and 10- and 15-year survival rates of 63% and 59%, respectively. Interestingly, the authors hypothesized that novel therapeutic approaches for enhanced treatment for PP may involve disrupting PP production of MUC-2, particularly by targeting the regulatory mechanisms underlying the transcriptional and post-transcriptional methods of mucin synthesis.

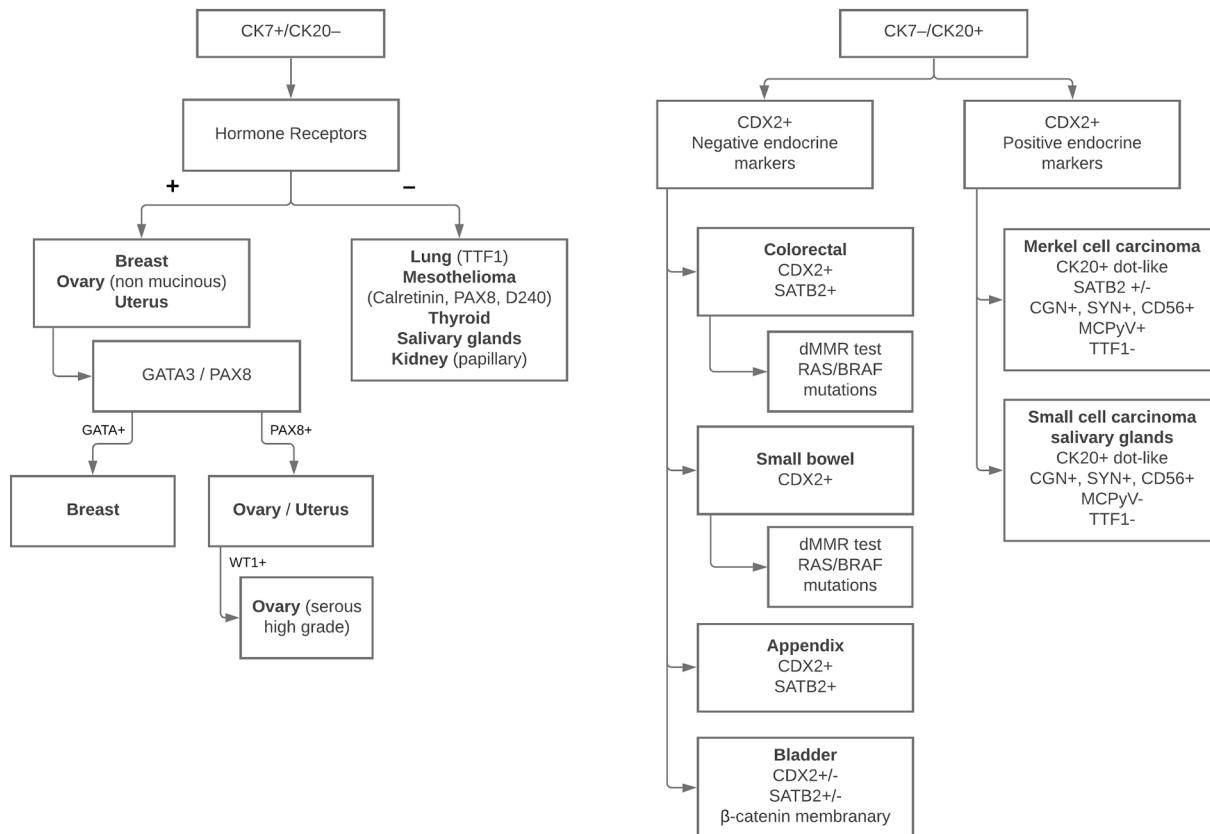
In contrast, the treatment of both peritoneal mesotheliomas and mucinous ovarian carcinomas are different with much worse outcomes [37,53]. Both are treated with platinum-containing regimens. Gorringe et al. [37] studied the therapeutic options for mucinous ovarian carcinoma. They reviewed 167 cases; 89 women (53%) were treated with surgery alone; 72/74 (97%) of cases had chemotherapy consisting of a platinum-based regimen; 61/74 (82%) also received a taxane as first line therapy. However, recurrent and high-grade mucinous ovarian carcinomas were uniformly resistant to conventional platinum / taxane therapy. Median survival for Stage III/IV disease was only 15 months. Nonetheless, there were no alternatively approved treatments available because of a lack of evidence for specific efficacy. Internationally co-ordinated attempts to develop mucinous ovarian carcinoma clinical trials to assess regimens designed for mucinous gastrointestinal cancers (since these regimens had been very effective) unfortunately met with little success.

As we see, the dramatic differences in prognosis and therapy for appendiceal mucinous tumors and associated PP, on one hand, and mucinous ovarian neoplasms and primary peritoneal mesotheliomas on the other, remind us again of our hypothesis that the cell of cancer origin still provides the most reliable roadmap to its diagnosis, biology and therapy.

#### Metastases of unknown origin (MUO)

Metastases of unknown origin (MUO) is another important cancer example that emphasizes the importance of defining the cell or tissue of

## Workup of MUO



**Fig. 4.** Flow chart schematic of IHC workup of MUO. Using a panel of IHC antibodies, based on select positive or negative immunoreactivity, a decision-making process is generated to determine site of origin [57]. However this approach usually is not definitive but yields only a differential diagnosis.

cancer origin as the most reliable roadmap to its diagnosis, prognosis (biology) and therapy. An understanding of MUO also illustrates how our paradigm-shifting hypothesis of exploiting similarities rather than differences between cancers and their cells of origin could lead to improved diagnosis, prognosis and therapeutic strategies.

MUO is also commonly referred to in the literature as carcinoma of unknown primary (CUP) [56–61]. The former term, however, is more accurate because the entity presents as metastatic disease, whose origin is unknown and not every case of MUO is a carcinoma. Regardless of which designation one uses, MUO or CUP, the entity is characterized by metastatic disease without an obvious primary site of origin (Fig. 3).

Within the past two decades, several studies have subdivided and classified MUO in different ways [56–61]. The first classification divided MUO into visceral MUO and lymph node MUO. According to Haskell et al. [56], “the prognosis may be quite good for patients with MUO limited to lymph nodes in the mid to high cervical, axillary, and groin areas. However, MUO in other lymph node areas is far more serious. Patients with MUO to visceral sites have a poor prognosis. However, metastases from some primary tumors are sensitive to chemotherapy and a limited search for these primary tumors should be undertaken. These tumors include leukemia-lymphoma, germ cell tumors, small cell carcinoma of the lung, adenocarcinomas of the breast, ovary, endometrium, thyroid, or prostate, and possibly adrenal carcinoma.” Interestingly, the most common visceral sites that MUO involve are the same sites that metastases of known origin involve, namely the liver and the lung (Fig. 3).

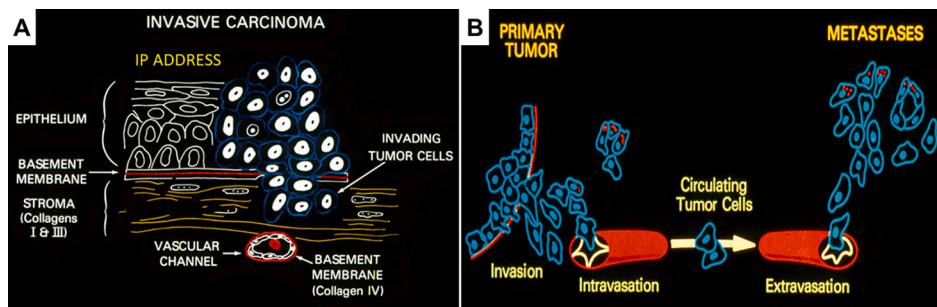
It has been estimated that MUO comprise between 2% – 15% of all metastatic cancers based on the definitions and diagnostic criteria which are ultimately applied and also form the basis of another classification

scheme. For example, many cases of MUO at the time of initial clinical presentation are eventually resolved during life by more sensitive imaging tests or after death by autopsy studies that determine the primary site of origin [58]. These latter MUO usually have small hard-to-detect primaries. It is not clear whether these small primary MUO behave the same way as overt primaries in terms of overall prognosis and response to therapy or whether they exhibit a different molecular signature with comprehensive genetic profiling than their overt primary counterparts [58].

Some MUO eventually turn out to emanate from a primary cancer that has spontaneously involuted, scarred or regressed through immune-mediated rejection (Fig. 3). Spontaneous involution with scarring is infrequently observed with testicular germ cell neoplasms and immune-mediated regression is observed with melanoma [56]. Still other MUO emanate from primaries at ectopic locations. For example, melanoma MUO can arise from neural crest neval cells present in ectopic locations, such as lymph node capsules and breast cancer presenting as an axillary lymph node MUO without a defined breast mass can actually arise from ectopic breast tissue located within the axilla (Fig. 3) [56].

Most authorities agree that every attempt should be made to determine the primary site in cases of MUO for reasons that include the fact that certain metastatic cancers with known overt primaries from certain sites are more susceptible to both empiric systemic chemotherapy, targeted directed therapy or immune therapy than metastatic cancers from other sites [58–61]. From this, if the same logic were applied to MUO, determining the primary site of origin would similarly determine susceptibility to therapy.

Historically, attempts to identify the primary site of MUO have been based on imaging studies, biopsy-based histopathological patterns,



**Fig. 5.** Schematic of MUO. MUO can be problematic in determining primary site of origin (A). Only a small fraction of tumor cells within a primary cancer actually metastasize (B) and therefore the metastases may not overall resemble the primary in terms of histology, biomarker expression or molecular profile. This may account for the present limitations in our ability to determine with certainty the primary site of origin. Yet the fact that MUO does, in fact, arise from some anatomical location raises the possibility that MUO carry with it site-specific biomarkers of that location, eg., an [“IP ADDRESS”] (A).

biopsy-based immunohistochemistry studies (IHC) and, more recently, molecular studies [61–72]. The latter approaches are, again, based on what has been learned from cases of metastases arising from known overt primaries.

IHC, in the past two decades, has been the standard in the workup of MUO and has been largely based on panels of antibodies which recognize tumor antigens. Interestingly, most of the tumor antigens exploited are antigens expressed within the tissue or cell of origin [57,60,62,70]. Decision-making flow charts based on Venn diagrams have emerged to guide the IHC workup of cases of MUO. A typical diagnostic decision-making flowchart is depicted (Fig. 4) [57]. Although the repertoire of IHC tests continues to expand, even under the most optimal situations, IHC only helps to resolve 30–40% of MUO cases. [58]. Very few IHC tests have greater than 90% sensitivity or 90% specificity, and, as a result, interpretation of the IHC panel usually gives a differential diagnosis rather than a precise primary site of origin determination (Fig. 4). This is probably because the antigens exploited represent a potpourri of site-specific and non-specific, and tumor-specific and non-specific biomarkers. IHC alone will not provide the roadmap needed to determine the primary tissue or cell of origin in our emerging era of personalized medicine.

For these reasons, specific genetic expression and epigenetic molecular profiles have emerged as characteristic of metastases and known overt primary cancers, and these have been applied to identifying sites of origin of MUO. [58,59,61,66]. Specific genetic expression profiles have been based on microarray and RT-PCR analyses [58]. A recent 92-gene RT-PCR molecular cancer classification assay (MCCA) has specifically been used [58,61]. Again this has been based on profiles generated by known primaries and their metastases and applied to MUO. Another molecular test that has been used is a genome-wide methylation profile exhibited by known primary neoplasms and their metastases and again applied to MUO [59]. These molecular approaches are superior to IHC in addressing the problem of MUO. It has been estimated that they resolve MUO at least 75% of the time [67]. However, one limitation is that analysis of the metastatic cancer produces a gene expression and methylation profile derived from both cancer cells as well as host cells embedded in the surrounding extracellular matrix, including inflammatory cells, fibroblasts and endothelial cells.

Interestingly, it has also been assumed or presumed that the molecular expression profile or the genome-wide methylation profile expressed by the primary tumor is reflective of the profile expressed by the cell or tissue of origin [59,61]. Despite existing support from studies that have examined subsets of expressed genes and methylation profiles in the primary tumors and that have compared them to normal tissue expression and normal tissue methylation profile datasets, these have been done more or less after the fact and after the expression profile of the known primary tumor was known and categorized [58,59,61]. A better approach might be to first determine the expression profile of normal organs and sites of cancer origin and work forward rather than backward from the cancer (Fig. 5). Recently, there has been interest in a class of genes termed transposable elements (TE) [73,74]. Although TEs have been studied for a considerable period of time, their tissue-specific

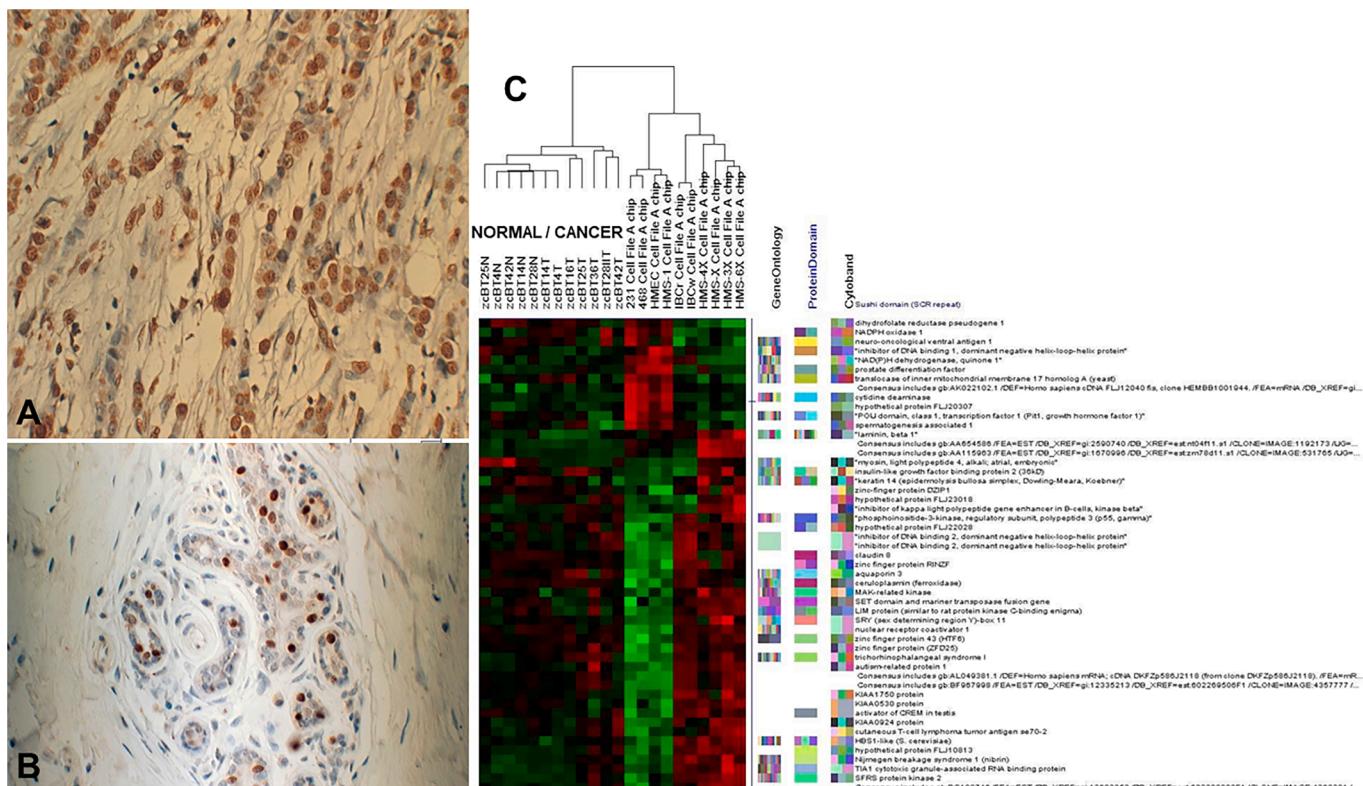
expression patterns have only recently been appreciated. Furthermore, transcriptome analysis of tumor-adjacent normal tissues reveals expression of similar transposable elements [73]. One class of particular TEs that have recently been observed in human RNA seq data is a class of long interspersed nuclear elements (LINE 1) (L1) [74]. These L1 elements are also observed within primary sites and primary cancers as well as within their metastases. This gives rise to our current belief that detecting these TE in MUO might provide the IP address needed to precisely locate the cell of cancer origin and work forward rather than backward (Fig. 5). Given the fact that RNA sequencing of single cells is now feasible, this approach would also eliminate the contribution of contaminating non-cancer cells. We intend to carry out such experiments in the near future. Demonstrating that L1 elements can be used to identify MUO would be further evidence that the cell or tissue of cancer origin still provides the most reliable roadmap to its diagnosis, prognosis (biology) and therapy.

In any molecular analysis, we must contrast the molecular cancer classification assay based on gene expression and / or methylation pattern of the primary tumor / site and the metastasis with so-called comprehensive genetic profiling which searches for acquired, potentially targetable molecular alterations in the cancer [58]. The former assay, at least theoretically, is designed to detect patterns of gene expression unique to the tissue of origin whereas the latter assay is really a type of molecular mutation profiling designed to detect altered oncogenes, suppressor genes or other potentially targetable molecular alterations acquired by the cancer. These latter alterations may be cancer-specific or present in many different cancer types. However they are not present within the primary cell or tissue of cancer origin. Examples are amplified Her-2/neu; mutated BRAF, EGFR, RET and BCRA1; and rearranged ALK and ROS1 [58]. These findings would seem to be at variance with our central hypothesis that the cell or tissue of cancer origin still provides the most reliable roadmap to its diagnosis, prognosis (biology) and therapy. However, these acquired and targeted molecular alterations discovered by comprehensive genetic profiling do not result in the same type of response among different tumors. For example, the dramatic response of metastatic melanoma with BRAF V600E-mutations is not observed at all in metastatic colorectal cancers with the same BRAF V600E-mutations [58], suggesting that a knowledge of the cell of origin may still provide the best roadmap to therapy even in the setting of subsequently acquired driver mutations. Finally, it should be emphasized that no treatments targeting acquired molecular alterations in metastatic disease, whether the disease is MUO or of known overt primary, result in cure.

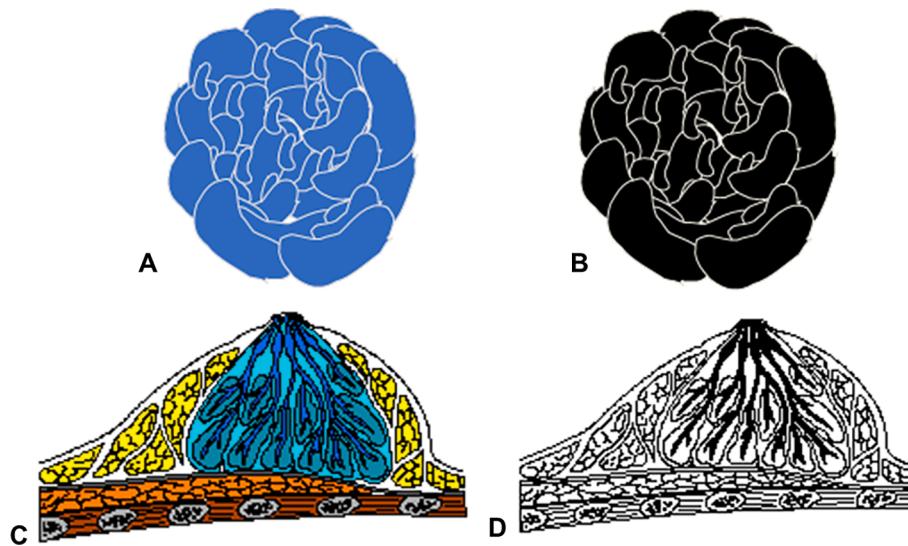
#### Cancers arising from dispensable organs (CAD)

The examples provided by PP and MUO illustrate cancers that, to a large extent, recapitulate the gene expression of their cell of origin. This recapitulation is not limited to a single gene but can involve multiple genes.

Metastatic breast cancer, for example, often expresses the estrogen receptor (ER), which is also expressed by its cell of origin within the



**Fig. 6.** Cases of metastatic and primary breast cancer. Metastatic breast cancer often exhibits estrogen receptor immunoreactivity (A) which reflects the estrogen receptor immunoreactivity of the cell of origin within the breast (B). But the similar gene expression is not limited to just one gene. Analysis of the entire transcriptome of individual cases of metastatic breast cancer [CANCER] reveals strong hierarchical clustering with the entire transcriptome of matched sets of normal breast [NORMAL] (C). (Portions of this composite figure are reproduced with permission from Fig. 3 of [75] Barsky SH. Myoepithelial mRNA expression profiling reveals a common tumor-suppressor phenotype. *Exp Mol Pathol* 2003;74:113–22. [https://doi.org/10.1016/S0014-4800\(03\)00011](https://doi.org/10.1016/S0014-4800(03)00011) by Agreement between Dr. Sanford Barsky and Elsevier via the Copyright Clearance Center).



**Fig. 7.** Schematic of the successful exploitation of this gene expression similarity. Breast cancer metastasis (A) can be successfully targeted and destroyed (B) targeting breast-specific genes in potentially dispensable organs, eg., breast. All the cells of origin which give rise to breast cancer in the normal breast, eg., epithelial cells (C) are also targeted and destroyed (D) without any dose-limiting toxicity because the breast, at least postmenopausally, is a dispensable organ.

breast (Fig. 6). But, it is not just the isolated gene that is expressed. It is the totality or near totality of gene expression which is similar between cancer and its cell of origin (Fig. 6). In a previous study [75], we conducted hierarchical clustering experiments on Affymetric chips which revealed a common mRNA expression profile between a given patient's

metastatic cancer and their normal breast. With this hierarchical clustering, a significantly distinct grouping of a given breast cancer with its tissue of origin was observed based on an analysis of 3200 genes (Fig. 6). Further analysis using a subset of 207 genes revealed tighter grouping. Also, rank ordering and hierarchical clustering of filtered genes showed

even tighter clustering between the breast cancers and their tissues of origin compared with different breast cancer cell lines, non-breast cancers and non-breast tissues.

Considering these and other previous studies, we would argue that it would be better to focus on the overall pattern of gene expression shared by the cancer and the cell of cancer origin. If we argue that our goal is to exploit the totality or near totality of similar gene expression between a given cancer and its cell of origin, then it would follow that the therapy targeting the cancer would be, in fact, the opposite of a “magic bullet”. It would follow that there would be no useful therapeutic index that we could exploit and that dose-limiting toxicity would be severely limiting — unless it did not at all matter that we destroyed the normal cell or organ of cancer origin (Fig. 7). Such would be the case in a number of dispensable organs or tissues that give rise to common cancers. Post menopausal women whose breasts give rise to most breast cancers do not need their ductal-lobular epithelium or stem / precursor cells. Similarly, men whose prostates give rise to cancer do not need their prostatic ductal-lobular epithelium. Colorectal carcinomas also arise in an organ which is dispensable. Melanomas arise in melanin-producing skin stem/progenitor cells whose destruction would only result in vitiligo. For both males and females, the most common cancers arise from organs or tissues that are dispensable. So, if we devised therapy which targeted the cancer based on its similarity of gene expression to its cell of origin, the opposite of a “magic bullet” approach, both the cancer and the cell or tissue of origin could be targeted non-discriminatorily but successfully (Fig. 7).

## Implications of the hypothesis

Targeting acquired molecular alterations in metastatic cancer does not result in cure. Instead, the cell of cancer origin, because it provides the most reliable roadmap to its diagnosis, prognosis (biology), and therapy, should be exploited. Treating metastatic cancer based on similarities of gene expression that identifies its cell of origin has already resulted in improved therapies. Targeting these similarities of gene expression further in CAD, because the therapy would not be dose-limiting, might even result in cure.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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