

● ● ● LYMPHOID NEOPLASIA

Comment on Massa et al, page 705

## Engineering Trojan-horse bacteria to fight cancer

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In this issue of *Blood*, Massa et al demonstrated that tumor-tropic bacteria engineered to express specific cancer-related antibodies can recognize and specifically destroy the malignant cells by infecting them with toxic cargo. The findings provide an essential step in establishing the novel approach of enlisting engineered bacteria that speak cancer's language to outsmart and defeat cancer.<sup>1</sup>

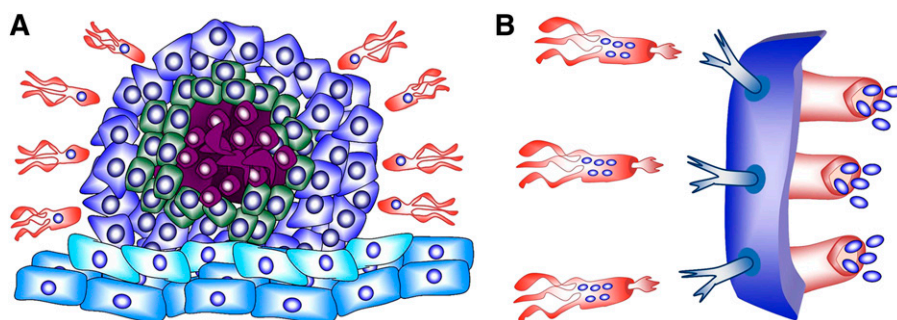
**D**espite much-improved knowledge of cancer biology, cancer continues to elude us and remains undefeated. Its most alarming aspects, namely, metastasis, relapse, and multiple drug resistance, are still inadequately understood and clinically insuperable.<sup>2-4</sup> It has become widely acknowledged that creative new ideas and out-of-the-box approaches to fight cancer ought to be explored. A promising new strategy is to recruit anaerobic bacteria that preferentially colonize and proliferate in the hypoxic environment of tumors.

Why bacteria and cancer? Increasing numbers of recent studies have revealed parallels between bacteria and cancer (eg, rapid proliferation, rapid development of drug resistance, and high phenotypic variability).<sup>4</sup> It has been conjectured that cancer shares so many features in common with bacteria because it represents an atavistic form of life, which ensued following a breakdown of regulatory pathways that evolved upon the transition from prokaryotes to eukaryotes.<sup>5</sup> This breakdown unlocked “ancient toolkits,” and thus cancer cells resort to more

fundamental strategies that have been developed by bacteria through the course of evolution. The underlying foundations of the bacteria-cancer parallels provide the rationale as to why wisely engineered bacteria can provide successful smart troops to fight cancer.

It has been shown that tumor-tropic bacteria can be recruited to assist the immune system to fight cancer.<sup>6</sup> Recent studies have demonstrated that the same capabilities that these smart organisms employ to trick the immune system can be turned to outsmart cancer. Injected *Salmonella* can cause melanoma cells to form gap junctions with adjunct immune dendritic cells.<sup>7</sup> Consequently, the dendritic cells use peptides transferred from the cancer cells to “teach” T cells to recognize and kill the tumor cells at the primary site and prevent metastasis formation.

The current studies of Massa et al<sup>1</sup> provide a crucial leap toward establishing this promising approach. The authors engineered nonvirulent tumor-tropic *Salmonella typhimurium* bacteria to selectively express CD20-targeting antibodies and demonstrated that the bacteria selectively infect CD20<sup>+</sup> lymphoma cells both in vitro and in vivo. Moreover, having been engineered to carry antitumor cargo (the herpes simplex virus thymidine kinase), the cancer-invading bacteria selectively transferred the harmful cargo and killed the cancer cells (see figure), so much so that the bacteria could successfully fight human lymphoma xenografts in mice lacking functional immune systems while protecting them from the dangerous virus. This vital proof-of-concept study kindles hope that the same strategy can be employed to target other types of cancer and selectively deliver and inject antitumor compounds directly into the cancer cells. Therefore, the new strategy paves the way to administer a range of potent drugs,



Schematic illustration of the new strategy to fight cancer using engineered Trojan-horse bacteria. (A) Illustration of the migration of cargo-carrying tumor-tropic bacteria toward a tumor. The outer cells (dark blue) are proliferating cells. The green cells represent dormant cancer stem cells (CSCs). Note that in real tumors the fraction of this subpopulation of cells is very low, and they can be widely scattered. The red cells are necrotic cells, and the light blue cells are stromal cells (see Ben-Jacob et al<sup>4</sup> for more details). (B) Illustration of cargo-carrying Trojan-horse bacteria engineered to express antibodies targeting the tumor-specific antigens. This recognition enables the bacteria to enter the cells and inject the antitumor cargo.<sup>1</sup>

which are not used currently because of their high toxicity to the body.

The new strategy also provides hope for successfully treating CSCs by engineering bacteria to express antibodies targeting specific markers of these cells. Although there is an ongoing debate regarding the terminology and the exact nature of CSCs, it is commonly agreed that tumors have small subpopulations of special tumorigenic cells with the ability of renewal and differentiation into a variety of cell types and cause tumor relapse.<sup>8</sup> These subpopulations pose a high risk because they are not detected by standard imaging methods, and since they can become dormant and thus successfully evade ordinary cancer treatments (chemotherapy, radiation therapy, and targeted/biological therapy). Development of specific therapies targeted at these transitional CSCs holds hope for improvement of survival and quality of life of cancer patients, especially for sufferers of metastatic disease. The advantage of the bacteria-based strategy is that the Trojan-horse bacteria engineered to express markers of both the tumor cells and the CSCs are expected to be able to find the dormant CSCs hiding within the tumor and attack them with newly discovered compounds effective against dormant cells.

An intriguing example of such a compound is salinomycin which is secreted by the *Streptomyces albus* bacteria.<sup>9</sup> This widely used agricultural agent has recently been shown to be highly potent against CSCs. As promising as it is, salinomycin cannot be used currently because of its high general toxicity. The Trojan-horse bacteria may be able to deliver the compound to kill the CSCs while protecting the rest of the body from the drug toxicity.

To conclude, building on the results reported by Massa et al,<sup>1</sup> we can envision recruiting engineered Trojan-horse bacteria to explore the body and find cancer cells. The engineered bacteria can then kill the cancer cells directly or expose them to the immune system and to other recruited bacteria engineered to kill them by acting together with the immune system. For example, it is now understood that most tumors at advanced stages are composed of several subclones. We can foresee the engineering of several Trojan-horse bacteria acting together, each able to better identify and target a specific clone. We may be seeing here the dawning of a new era of biological

cyberwarfare on cancer, in which we will enlist bacteria to fight with the immune system and defeat cancer with minimal side effects to the patients.

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## ● ● ● MYELOID NEOPLASIA

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# Arginase-mediated “field” defects in AML

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In this issue of *Blood*, Mussai et al report on the ability of acute myeloid leukemia (AML) blasts to inhibit the immune system and hematopoiesis through aberrantly high levels of arginase II activity.<sup>1</sup> AML is a clinically, genetically, and epigenetically heterogeneous disease.<sup>2</sup> Both epigenetic and genetic aberrations in AML blasts have been used to understand mechanisms of leukemia growth and predict patients' outcomes, as well as assign them to different treatment approaches. However, the majority of patients treated with chemotherapy and/or molecular targeting agents continue to die of their disease. Stem cell transplantation (SCT) has been proven effective in curing AML by allowing for the administration of otherwise myeloablative and toxic doses of chemotherapy and for reconstitution of an effective immune system from the donor graft that, in turn, contributes to the eradication of resistant clonal cell subpopulations. These populations may include those with high self-renewal ability: the so-called leukemia stem (or initiating) cells. However, even following SCT, patients may relapse and die of their disease, underlying the complexity of AML biology and of the mechanisms of disease resistance.

**T**o date, the main focus of AML studies has been on the genetic and epigenetic features of the blasts and how these characteristics relate to mechanisms leading to the arrest of hematopoietic differentiation and the ability to proliferate and survive even after administration of cytotoxic compounds. However, an increasing number of studies now emphasize the biologic significance and potential clinical relevance of the microenvironment in AML.<sup>3,4</sup> The findings by Mussai et al<sup>1</sup> further support this view by showing that aberrant arginase II activity

contributes to leukemogenesis via suppression of T lymphocyte activity through polarization of monocytes into a suppressive M2-like phenotype and direct inhibition of the hematopoietic activity, which in turn may contribute to pancytopenia and marrow failure observed in AML. The role of arginase I in mechanisms of immunosuppression through myeloid-derived suppressor cells has been previously recognized,<sup>5,6</sup> whereas the activity of arginase II leading to an aberrant microenvironment is an original observation that reemphasizes the potential of myeloid