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Cancer Immunology Research

Meeting Report

Koch Institute Symposium on Cancer Immunology and Immunotherapy

Adam Drake[1], Nikhil S. Joshi[1], Gregory L. Szeto[1], Eric F. Zhu[1,2], Herman N. Eisen[1,3,5], and Darrell J. Irvine[1,4,5,6,7]

Abstract

The 12th annual summer symposium of The Koch Institute for Integrative Cancer Research at MIT was held in Cambridge, Massachusetts, on June 14, 2013. The symposium, entitled "Cancer Immunology and Immunotherapy," focused on recent advances in preclinical research in basic immunology and biomedical engineering and their clinical application in cancer therapies. The day-long gathering also provided a forum for discussion and potential collaborations between engineers and clinical investigators. The major topics presented included (i) enhancement of adoptive cell therapy by engineering to improve the ability and functionality of T cells against tumor cells; (ii) current therapies using protein and antibody therapeutics to modulate endogenous antitumor immunity; and (iii) new technologies to identify molecular targets and assess therapeutic efficacy, and devices to control and target drug delivery more effectively and efficiently. *Cancer Immunol Res; 1(4); 217–22. ©2013 AACR.*

Introduction

Recent advances in cancer immunotherapy, the product of many years of basic and translational research, are harbingers of a new age of cancer treatment in which immunotherapy will likely play an increasing role in the management and control of cancer. The 12th annual summer symposium of The Koch Institute for Integrative Cancer Research at MIT (Cambridge, MA) focused on this timely topic, covering preclinical and clinical advances in cancer immunology and immunotherapy. In keeping with the interdisciplinary biology engineering fusion of the Koch Institute, this day-long meeting included presentations by clinical researchers on cancer therapies, by immunologists on the fundamentals of cancer immunobiology and by engineers on the development of new therapeutic and diagnostic technologies for assessing and treating cancer.

Enhancing T-cell Therapy of Cancer

T cells are potentially ideal antitumor effectors, as they can seek out and eliminate tumors or micrometastases in any tissue and provide long-lived protection against recurrence. To this end, adoptive cell therapy (ACT) has been developed using purified autologous patient-derived tumor-reactive T cells that are expanded *in vitro* and then reinfused into the patient (1, 2). T-cell therapy can improve outcomes for patients with late-stage melanomas, eradicating tumor masses in multiple sites and providing long-term regression (3). Although such striking responses were found in only a minority of patients in early trials, recent attempts to improve ACT by engineering antitumor T cells have significantly increased the effectiveness of ACT in several tumor types, and T-cell engineering may provide the means required to eliminate advanced tumors in a higher proportion of patients with cancer. These efforts can be divided into those focused on increasing the ability of T cells to recognize tumor cells and those focused on improving the functions of the reinfused T cells.

Engineering tumor-specific T cells

Dr. Carl June of the University of Pennsylvania (Philadelphia, PA) reported on recent advances in engineering chimeric antigen receptors (CAR): T-cell receptors (TCR) in which the MHC-binding loops are replaced by antigen-binding regions from single-chain antibodies that target molecules on cancer cells (4). With antibodies serving as antigen-recognizing receptors on T cells, these CAR T cells acquire the ability to respond to a wider range of epitopes and with higher affinity than the T cells' natural receptor (TCR). Dr. June's team generated CD19-specific CAR T cells by transducing patient T cells with lentiviral vectors expressing anti-CD19 CARs and infused these autologous cells into patients with chronic lymphocytic leukemia (CLL) and acute lymphoblastic leukemia (ALL; refs. 5–7). This approach provides a number of advantages for ACT, including the ability to generate tumor-specific cells from any T-cell subset and target an abundant and conserved surface antigen expressed by tumors of most CLL and ALL patients. In addition, the CAR design can be tailored to improve T-cell function, as Dr. June showed that the addition of signaling domains from costimulatory molecules (e.g., 4-1BB) allowed the engineered T cells to expand over extended duration in cell culture.

Therapeutically, the transfer of autologous anti-CD19 CAR-expressing T cells into CLL and ALL patients resulted in

Authors' Affiliations: [1]Koch Institute for Integrative Cancer Research, Departments of [2]Chemical Engineering, [3]Biology, [4]Biological Engineering, and [5]Materials Science & Engineering, Massachusetts Institute of Technology; [6]Ragon Institute of MGH, MIT, and Harvard, Cambridge, Massachusetts; and [7]Howard Hughes Medical Institute, Chevy Chase, Maryland

Corresponding Author: Darrell J. Irvine, Massachusetts Institute of Technology, Biological Engineering, MIT Room 8-425, 77 Mass. Avenue, Cambridge, MA 02139. Phone: 617-452-4174; Fax: 617-452-3293; E-mail: djirvine@mit.edu

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approximately 59% and 90% response rates, respectively, albeit the number of treated patients to date is still small; importantly, many responders remained tumor free for up to 36 months after treatment. The CD19 CAR T cells proliferated 100- to 1,000-fold in all patients for over 2 years. Persistence of functional CAR T cells in these patients was suggested by the finding that normal B cells (which also express CD19) remain at low levels even 36 months after treatment, as the normal B cells are expected to recover if the CAR T cells lost function. In some patients, the antitumor response was associated with significant interleukin (IL-6)–mediated toxicity, which was ameliorated with anti-IL-6 receptor antibodies. Dr. June showed results from one patient whose pretreatment tumor population contained a mixture of $CD34^+CD19^+$ and $CD34^+CD19^-$ cells. After ACT treatment with CD19 CAR T cells, the $CD34^+CD19^+$ cells were eliminated, but the $CD34^+CD19^-$ cells persisted. This example illustrates the limitation in using monoclonal T cells in ACT and suggests that targeting multiple antigens might prevent tumor escape. He closed with a discussion of challenges in scaling up ACT to broader patient populations (8) and of additional strategies to enhance T-cell therapy via "smart" CARs under the control of genetic Boolean-gated logic circuits that would allow T cells to discriminate between tumor and bystander cells. Similar promising results from a clinical trial conducted at the Memorial Sloan-Kettering Cancer Center (New York, NY) on adults with ALL were reported recently, suggesting that CARs might have a major role in the treatment of B-cell malignancies (9).

Improving the function of antitumor T cells

Engineering efficient tumor recognition by T cells is necessary but not sufficient for successful ACT, as transferred T cells must also maintain their effector functions within the tumor microenvironment. Immunosuppressive cells, cytokines, and inhibitory ligands within tumors can inhibit even very potent antitumor immune responses, and furthermore, solid tumors do not provide costimulatory signals that support durable T-cell responses. Taking an engineering approach to address this problem, Dr. Darrell J. Irvine of Koch Institute described a strategy for attaching backpack-like nanoparticles onto antitumor T cells *ex vivo*, and the successful adoptive transfer and homing of these engineered T cells to tumor tissue. The number of nanoparticles per T cell was carefully controlled so as not to interfere with the ability of the engineered T cell to interact with an antigen-presenting or tumor cell. This approach allowed both biologics and small-molecule drugs to be carried by T cells into tumors. For example, when IL-15 was loaded onto nanoparticles, the proliferation and survival of T cells within the tumor increased, greatly amplifying the antitumor responses compared with systemic cytokine administration (10).

Nanoparticles attached to T cells localized to the immunologic synapse when T cells interacted with tumor cells; Dr. Irvine showed that this approach could be used to focus small-molecule inhibitors of the phosphatases Shp1 and Shp2 to the synapse to protect T cells from suppressive signaling in the tumor microenvironment (11). Dr. Irvine also described the next-generation strategy of "targeted nanoparticles," which, after being injected into patients, could attach directly onto tumor-specific T cells *in vivo*, obviating the need for *ex vivo* manipulation and introducing the possibility of repeated "rearming" of T cells *in vivo* (12). Overall, these strategies provide a means to deliver drugs in an autocrine or paracrine fashion to tumors, lymphoid organs, or other tissue sites guided by the specificity and phenotype of the carrier lymphocyte.

Antibodies and Protein Therapeutics for Immunomodulation

With the success of the anti-CTLA-4 antibody ipilimumab, enhancement of endogenous antitumor immune responses through antibody-mediated strategies of immunomodulation has become a major focus in preclinical and clinical studies (13). Three of the talks at the symposium focused on immunomodulation of antitumor immunity using protein and antibody therapeutics.

Fundamental immunology and clinical promise of modulating the PD-1/PD-L1 axis

Dr. Lieping Chen of Yale University (New Haven, CT) discussed the PD-L1 pathway of immunosuppression and its role in tumor immunology. PD-L1 (also called B7-H1) is a ligand for T-cell surface receptor PD-1 (CD279), and the binding of PD-L1 to PD-1 suppresses T-cell function (14). Dr. Chen showed that in normal human tissues, PD-L1 expression is generally absent, but all nucleated cells can be induced to express PD-L1 through the IFN family of cytokines, as well as some TLR ligands (14). PD-L1 knockout mice exhibit very little phenotypic differences from wild-type mice, without any indications of autoimmunity (15). Thus, it would appear that PD-L1 expression is only activated as a suppressive mechanism during the course of infection or inflammation to dampen the tissue-damaging effects of overly active T cells. However, by expressing PD-L1, tumors can exploit its immunosuppressive activity, leading to the direct exhaustion of tumor infiltrating tumor-specific T cells. Work by Dong and colleagues has shown that P815 mouse mastocytomas engineered to overexpress PD-L1 showed a slight growth advantage compared with wild-type tumors. However, these $PD\text{-}L1^+$ tumors were resistant to therapy based on transfer of activated T cells (16). Further mechanistic studies show that PD-L1 signaling can result in various immunosuppressive effects, such as IL-10 production, T-cell tolerance, exhaustion, apoptosis, and the modulation of T-regulatory cells (Treg) and antigen-presenting cells (14). PD-L1 expressed on tumor cells can also act as a receptor via PD-1 engagement, leading to the induction of antiapoptotic mechanisms within the tumor (17).

Dong and colleagues have observed PD-L1 expression in a variety of human cancers ref. 16. Consistent with these observations, a phase I clinical trial to assess the safety, dose, and activity of the fully humanized anti-PD-1 monoclonal antibody nivolumab (BMS-936558) has shown promising results in multiple advanced cancers (18). A cohort of 296 patients for whom conventional therapy had failed were treated, with many objective responses observed, particularly in patients with

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melanoma, renal cell carcinoma, and some lung cancers (non–small cell lung cancer; ref. 18). Importantly, many patients experienced a striking decrease or complete regression of tumor masses after several cycles of anti-PD-1 nivolumab therapy (18, 19). Even new metastatic nodes that developed in some patients experienced regression under continued treatment. Overall, anti-PD-1 treatment was well tolerated, and many responses were highly durable, with patients living for years after the end of the trial (19).

Checkpoint blockade with anti-CTLA-4

The anti-CTLA-4 antibody ipilimumab was the first U.S. Food and Drug Administration (FDA)–approved antibody intended to block regulatory signals suppressing antitumor T-cell responses ("checkpoint blockade"). In a phase III randomized trial led by Dr. Stephen Hodi of Harvard Medical School and the Dana-Farber Cancer Institute (Boston, MA), HLA-A0201*-positive patients with metastatic melanoma, who had received previous therapy, were given ipilimumab alone, a peptide vaccine targeting a melanoma antigen (gp100), or the two in combination. Hodi and colleagues showed that ipilimumab was able to substantially increase median overall survival with or without the peptide vaccine, compared with those treated with the peptide vaccine alone (20). Strikingly, in some patients the effects of anti-CTLA-4 were long term, with patient survival lasting from many months to years.

Dr. Hodi and colleagues endeavored to further increase the efficacy of anti-CTLA-4 therapy. In murine models, CTLA-4 blockade combined with GVAX, a vaccine of tumor cells engineered to express granulocyte macrophage colony–stimulating factor (GM-CSF), showed potent synergistic effects toward tumor killing as well as the development of immunologic memory (21). On the basis of these encouraging results, a small clinical trial was conducted to assess the efficacy of ipilimumab on 14 patients with stage IV melanoma who had previously received GVAX. Hodi and colleagues found dense infiltrates of CD4^{+}, CD8^{+}, and CD20^{+} cells in biopsies of the treated tumors, suggesting a concerted cellular and humoral immune response was in play. Necrosis of the treated tumor was observed, as well as immune-mediated vasculopathy, indicating that the immune system was able to distinguish between tumor blood vessels and the blood vessels of normal tissue. He also presented data that correlated the extent of necrosis with the ratio of CD8/Foxp3 found in the various specimens, implicating a shift from a Treg-enriched to an effector-T-cell–enriched microenvironment in responding tumors. Dr. Hodi also discussed alternative combinatorial approaches in combination with ipilimumab, as GVAX is not readily available for all patients.

Synergistic modulation of innate and adaptive immunity

Complementary to checkpoint blockade therapies, Dr. Dane Wittrup of the Koch Institute discussed strategies to synergistically direct innate and adaptive immunity against tumors via combination immunomodulatory biologics. In preclinical studies, Wittrup and colleagues showed that a combination therapy comprising an engineered cytokine construct with extended circulation time and tumor-targeting antibodies elicited marked suppression of tumor growth in the aggressive B16F10 melanoma model.

They showed that both adaptive and innate immune cells were essential for efficacy of this therapy, suggesting a possible cross-talk between the two arms of the immune system that can be induced for potent tumor control. Inflammatory cytokines were highly elevated within the tumor mass, again providing evidence that a strong tumor-killing response was being generated. Although the majority of tumors grew out after cessation of the combination therapy, Wittrup and colleagues found that a triple combination that included the engineered cytokine construct, tumor-targeted antibody, and adoptive transfer of melanocyte antigen-specific (Pmel-1) CD8^{+} T cells led to robust tumor control that persisted long after the cessation of therapy in all mice and was accompanied by prominent vitiligo. This result suggests that, although the innate immune response can synergize with an adaptive immune response, an extremely vigorous tumor-specific CD8^{+} T-cell response is likely still necessary for complete tumor elimination.

Vaccines against Cancer

Vaccines are one of the most impactful biomedical advances of the 20th century, essentially eradicating morbidity and mortality for numerous major infectious diseases. There is high interest in cancer vaccines because of their potential to deliver both therapeutic and preventive intervention with specific, targeted responses and potentially long-lived protection through immunologic memory. The hepatitis B virus vaccine, developed originally to prevent liver cell infection, has led to a greatly reduced incidence of hepatocellular carcinomas worldwide. Major clinical breakthroughs in the development of new anticancer vaccines include the therapeutic prostate cancer (Provenge; Dendreon) and prophylactic cervical cancer (Gardasil; Merck and Cervarix; GlaxoSmithKline) vaccines. The former is a form of cell therapy, relying on isolation, antigen loading, and reinfusion of autologous antigen-presenting cells (APC); the latter uses a more traditional vaccine approach by targeting a cancer-causing pathogen (human papillomavirus; HPV) using a protein subunit as immunogen.

Tissue immunology of therapeutic HPV vaccines

Approximately 20% of human cancers can be attributed to infectious pathogens; HPV causes a high incidence of cancers at multiple sites, including the cervix and oropharynx. Dr. Connie Trimble of the Johns Hopkins Medical Institute (Baltimore, MD) described efforts to characterize the clinical immune response to therapeutic vaccines with the goal of eliminating precancerous cervical HPV lesions. Trimble and colleagues developed a plasmid DNA vaccine that cured established HPV strain E7-expressing tumors in mice. On the basis of successful preclinical results, a phase I clinical trial for patients with high-grade cervical intraepithelial neoplasia (CIN2/3) HPV16^{+} lesions preresection was conducted with the DNA vaccine (22). Results of the phase I trial were similar to those from other vaccine trials targeting

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preinvasive CIN: The vaccine elicited low-level circulating tumor-specific T-cell responses (peak ~20 cells per million in the blood; refs. 22, 23). However, detailed examination of immune infiltrates in the cervical tissues revealed that immune cells were localized to stromal regions adjacent to (but outside) persistent neoplastic lesions (24); importantly, immune cell infiltration into the lesion correlated with tumor regression. Cervical T cells were found to express the homing marker $\alpha_4\beta_7$, colocalizing with expression of its ligand (MAdCAM-1) on vascular endothelium (24). MAdCAM-1 was present in normal mucosa but absent on lesional vasculature (24).

Delving deeper into the finding that vaccines eliciting negligible systemic T-cell responses may have substantial effects in the cervical lesions, Dr. Trimble described additional features of the immune response that developed in cervical tissues. There appear to be striking changes in these tissues after vaccination, including increased numbers of recruited proliferating $CD8^+$ T cells with a T_H1 phenotype. These findings motivated ongoing studies in which DNA vaccines are being boosted with viral vector-based constructs and are also augmented with topical application of the Toll-like receptor-7 agonist imiquimod to promote a tissue-based immune response.

Devices to program antitumor immunity *in vivo*

Approval of Provenge for prostate cancer treatment was a big step forward in the development of cancer vaccines, but Provenge is a complex and expensive treatment based on *ex vivo* manipulation of autologous APCs. Dr. David Mooney of Harvard University described recent efforts to engineer APC differentiation, activation, and antigen loading directly *in vivo* for enhanced tumor vaccines. Using techniques he established for regenerative medicine, Dr. Mooney designed polymeric implants loaded with GM-CSF and CpG to sequentially recruit circulating dendritic cell (DC) precursors and expose them to TLR ligands ("danger signals") and tumor antigens within the polymer matrix that facilitate priming and differentiation. Subsequently, these *in situ*-primed DCs migrate to lymph nodes. The polymeric scaffolds can be loaded with the cytokine GM-CSF, using different doses and controlled release kinetics to recruit the desired numbers of DCs ref. 25. He showed that CpG and GM-CSF are synergistic, increasing DC antigen-uptake in mice, with more than 1 million antigen-bearing DCs migrating to lymph nodes in 1 week while attenuating the activity of Tregs (25, 26).

The inclusion of tumor lysate in the implant generated a significant antigen-specific effector $CD8^+$ T-cell response at both the implant site and in the spleen (27). Survival positively correlated with the numbers of recruited $CD8^+$ and plasmacytoid DCs (27); the implants were ineffective in $CD8^+$ DC knockout mice. Highlighting the synergistic effect of combination therapy, Dr. Mooney showed that immune checkpoint blockade antibodies dramatically enhanced vaccine efficacy. He also emphasized the modularity of the platform, further broadening application to other cancers (glioblastoma) and diseases (autoimmunity, transplant rejection). Dr. Mooney ended the presentation by describing recent FDA approval of the first clinical trial using this implant vaccine approach as a therapeutic melanoma vaccine; the trial will be conducted in collaboration with Drs. Glenn Dranoff and Steven Hodi at the Dana-Farber Cancer Institute.

New Technologies and Cancer Immunotherapy: Immunobiology and Technology

The recent clinical advances in cancer immunotherapy have been underpinned by our developing understanding of cancer immunobiology. Moving forward, new classes of molecular targets need to be identified, as well as more effective reagents against existing targets and better identification of patients who will benefit from each therapeutic intervention. Improvements in animal models, development of new analytic technologies, and more detailed assessment of the immunologically relevant changes that differentiate tumor from healthy tissue are central to this process. Jianzhu Chen, Chris Love, and Robert Schreiber addressed advances in these areas.

Modeling cancer with humanized mice

Animal models that contain human cells and tissues as targets may provide a better preclinical system to evaluate antibodies and other proteins as "drugs" for human diseases. Dr. Jianzhu Chen of the Koch Institute presented the humanized mouse model as one solution to better preclinical testing. In this approach, immunocompromised mice are reconstituted with human hematopoietic stem cells (HSC) to generate mice with a human immune system (humice; refs. 28, 29). HSCs can be cultured (30) and infected with a lentiviral or retroviral vector to generate transformed cells that will effectively model human leukemias when transferred into immunodeficient mice (31).

Dr. Chen illustrated this approach first with chemotherapy-resistant pre-B-cell acute lymphoblastic leukemia created by overexpressing Myc and BCL2 (32). Using this humanized mouse model, he showed that the combination of cyclophosphamide and anti-human CD52 antibody (alemtuzumab) was synergistic; this chemo-immunotherapy regimen eradicated residual disease in the bone marrow (Pallasch et al.; manuscript submitted). A second humanized mouse tumor model described was the EBV-induced human B-cell lymphoma (Oo et al.; manuscript submitted ref. 33), which was used to examine the efficacy of the therapeutic antibody, rituximab (anti-CD20), and which prolonged the survival of the treated humice with B-cell lymphoma. Finally, Dr. Chen reported the reconstitution of other tissues with matched human stem cells. Using appropriately selected stem cells from diverse tissues, mice were reconstituted with humanized liver, pancreas, and kidney tissues (34–36). Combined with the lentiviral transduction technology, this paves the way for the corresponding human solid tumors to be developed in these humice with a matched human immune system. These humanized mouse models offer considerable promise not only for improving preclinical assessment of therapeutics but also for personalized medicine in which the tumor and immune cells from the same patient could be analyzed.

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Analyzing human immunity at the single-cell level

In addition to new animal models, novel analytic technologies significantly advance our understanding of cancer. Dr. J. Christopher Love of the Koch Institute talked about the combination of modern techniques for fluid handling, microscopy, and a technology known as microengraving, in which individual or small numbers of cells are isolated within an array of subnanoliter wells for characterization of multiple live cell functions over time (37). A combination of image cytometry and microengraving allows cells to be phenotyped for up to 12 cell surface molecules and four to five secreted analytes, following which individual cells can be retrieved for DNA/RNA analysis or subsequent culture (38, 39). This cell-by-cell readout allows detailed analysis of the small number of cells obtained from tumor samples such as biopsies or longitudinal studies of single cells.

Dr. Love showed that this approach can reveal unappreciated details of immune cell function at the single-cell level. Examining human T-cell cytokine secretion following activation, he showed that T cells tend to produce cytokines sequentially and that different subsets of T cells show predictable patterns of cytokine evolution over time (40, 41). The dynamics of leukocyte–tumor cell interactions can also be observed by mixing the two populations in microwells. This was illustrated by recent work showing that there is no short-range cooperativity between NK cells killing target cells (42).

Defining antigens that support immune-mediated tumor destruction

Dr. Robert Schreiber of Washington University (St. Louis, MO) described studies focusing on factors that determine whether the immune system rejects or fails to reject nascent tumors. He reviewed the concept of cancer immunoediting whereby tumors change over time in response to immune pressure (43). Initially, immune responses may eliminate tumors or result in transitions to a steady state balancing tumor cell proliferation and cell death, and often resulting in tumors that escape immune pressure by a combination of factors including antigen loss and the development of an immunosuppressive microenvironment within the tumor mass (44). In support of the immunoediting model, Dr. Schreiber showed recent DNA sequencing analyses of nascent methylcholanthrene (MCA)-induced tumors, in which he identified a mutated self-antigen that allowed T-cell–mediated rejection of MCA-derived sarcomas (45).

Following on this key finding, Dr. Schreiber reported ongoing work applying this approach to identify antigens that can be targeted to reject tumors that normally would not be eliminated by the endogenous immune response. CTL clones were isolated from mice that rejected progressing tumors following treatment with anti-CTLA-4 or anti-PD-1/anti-PD-L1 antibodies. The clones were then tested for recognition of mutant epitopes identified in the tumors by DNA sequencing. These analyses identified a single major epitope that appeared to be targeted by multiple distinct CTL clones which, in preliminary data, appeared to support rejection of these tumors when the epitope was given as the key component of a peptide vaccine. This approach might be used in the future to identify effective vaccine antigens for an individual patient's tumor.

Conclusions

About a hundred years ago, immunologists realized that serum antibodies could be elicited against not only bacteria and their toxins but also against a seemingly unlimited variety of substances. This realization may have led Paul Ehrlich to propose that antibodies to cancer antigens could suppress or eliminate cancers. This simple concept has now evolved in parallel with our understanding of the biology of cancer into an array of strategies aiming to invoke both innate and adaptive immunity against tumors. These advances in cancer immunology and immunotherapy reported at the Koch Institute summer symposium include research in clinical investigation, applied engineering, and basic immunology. These advances show that the field of cancer immunotherapy has finally begun to yield success after many years of therapies with limited efficacy. A number of challenges lie ahead in each area described here, but there is much reason for optimism that cancer immunotherapy can contribute not only to the armamentarium against cancer, but may also significantly increase patient survival and quality of life under conditions in which traditional therapies fail.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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