PI3K/Akt/mTOR signaling pathway and targeted therapy for glioblastoma

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ABSTRACT

Glioblastoma multiform (GBM) is the most common malignant glioma of all the brain tumors and currently effective treatment options are still lacking. GBM is frequently accompanied with overexpression and/or mutation of epidermal growth factor receptor (EGFR), which subsequently leads to activation of many downstream signal pathways such as phosphatidylinositol 3-kinase (PI3K)/Akt/rapamycin-sensitive mTOR-complex (mTOR) pathway. Here we explored the reason why inhibition of the pathway may serve as a compelling therapeutic target for the disease, and provided an update data of EGFR and PI3K/Akt/mTOR inhibitors in clinical trials.

INTRODUCTION

Glioblastoma multiform

Glioblastoma multiform (GBM), WHO grade IV, is the most common and aggressive glioma of all primary brain tumors, exhibiting a high rate of recurrence and poor prognosis due to the invasive nature of the tumor [1, 2]. Considering the location and diffusely infiltrating nature of the tumor, complete surgical resections are challenging. Standard therapy for GBM is radiation plus the chemopreventive agent temozolomide (TMZ). The cytotoxicity of TMZ is thought to be primarily due to alkylation of DNA hence leading to DNA damage and tumor cell death [3]. However, the activation of PI3K/Akt/mTOR pathway leads to the development of drug resistance thereby dampening the therapeutic effect of TMZ [4]. The five year survival rate for glioblastoma is less than 5% in adults [5-7]. The occurrence of GBM is frequently associated with molecular changes in epidermal growth factor receptor (EGFR) and phosphatidylinositol 3-kinase (PI3K)/Akt/rapamycin-sensitive mTOR-complex (mTOR) pathways. The frequency of genetic alterations such as overexpression EGFR, activating mutations of PI3CA (p110) or PIK3R1 (P85), or loss of PTEN expression has been estimated to around 88% [8-12]. GBM patients with an activated PI3K/Akt/mTOR pathway also have poor prognosis than patients without oncogenic activation of the pathway [13]. Therefore, inhibitors targeting EGFR and PI3K/Akt/mTOR pathway have emerged as potential treatment for GBM [14-18]. Currently, a series of inhibitors targeting EGFR and PI3K/Akt/mTOR pathway are evaluated in preclinical and clinical studies as single agent or in combination with the traditional treatment [19]. It is of particular interest to explore whether those inhibitors are effective to restore the therapeutic sensitivity.

EGFR and PI3K/Akt/mTOR signal transduction pathway

EGFR is a type of receptor tyrosine kinases (RTKs), playing a central role in cell division, migration, adhesion, differentiation and apoptosis [20, 21]. EGFR comprises of extracellular ligand binding domain, transmembrane domain and intracellular tyrosine kinase domain. Upon binding to various of ligands, such as EGF and
TGFα, EGFR is activated through homodimerization or heterodimerization on the cell surface and subsequently leads to the phosphorylation of its intracellular tyrosine kinase domain [22]. The activation of EGFR results in activation of multiple downstream signal transduction pathways such as PI3K/Akt/mTOR pathway [23].

Members of the PI3K family are lipid kinases involved in multiple cellular process, including proliferation, differentiation, migration, metabolism and survival [24]. PI3K is generally classified into three classes according to their substrate specificity and subsequent homology, among which, the class I is most vital to the tumorigenesis. Class I consisted of a catalytic subunit p110 (α, β, γ) and a regulator subunit p85. A fourth p110 isoform (p110δ) is paired with the p101 regulatory subunit in class IB PI3Ks. Upon ligand binding, phosphorylated tyrosine residing in activated RTKs will bind to p85. The subsequent conformation change will release the catalytic subunit p110 [25], where activated p110 phosphorylated the phosphatidy-linositol-3, 4-bisphosphate (PIP2) into the second messenger phosphatidylinositol-3, 4, 5-bisphosphate (PIP3). This reaction can be reversed by the PI3K antagonist PTEN (phosphatase and tensin homolog deleted on chromosome ten) [26]. Subsequently, PIP3 will recruit the downstream Akt to inner membranes and phosphorylates Akt on its serine/threonine kinasesites (Thr308 and Ser473) [27, 28]. Activated Akt is involved in the downstream mTORC1 mediated response to biogenesis of protein and ribosome.

In PI3K pathway, mTOR acts as both a downstream effector and an upstream regulator [29, 30]. mTOR resides in rapamycin-sensitive mTOR-complex (mTORC1) and a rapamycin-insensitive complex (mTORC2) [31, 32]. The activated Akt inhibits tuberous sclerosis complex (TSC) 1/2 activity, thereby initiate the mTORC1-mediate signaling pathway, involving in the phosphorylation of ribosomal protein S6 kinase (pS6k), eukaryotic initiation factor 4E (eIF4E) and eukaryotic initiation factor binding protein 1(4EBP1), which participate in protein translation, ribosome biogenesis as well as cell growth [33, 34]. The mTORC2 phosphorylates Akt at Ser-473, and then further takes part in cell survival, metabolism, proliferation, and cytoskeletal organization [31, 35]. Within PI3K signaling pathway, another important molecule is PTEN. As clinical research revealed, the EGFR or PTEN mutation would lead to continuous activation of PI3K/Akt/mTOR signaling pathway, thereby contributing to the tumorigenesis and cancer therapy resistance (Figure 1).

**Figure 1: Schematic representation of the PI3K/Akt/mTOR signaling pathway.** Upon relevant ligand binding, RTK, such as EGFR, is activated and subsequently inducing a series of cascade reaction. First, the regulator subunits of PI3K, p85, dimerize and release its catalytic subunit p110. p110 enables the membrane protein PI2P to phosphorylate into PI3. PI3 begins to recruit the downstream Akt to inner membranes and phosphorylated the serine/threonine kinase (Thr308 and Ser473) sites by phosphoinositide-dependent kinase 1/2 (PDK1/2). Activated Akt is involved in the downstream mTORC1 mediated response to biogenesis of protein and ribosome. Besides that, activated Akt is also involved in the regulation of cell cycle and pro-apoptotic and anti-apoptotic factors mediated choices of cell apoptosis and survival. Additionally, it is also involved in the NFkB/MDR1 mediated drug resistance.
| Drug | Targets | Combination Partner | Patient group | Phase | State | Trail ID |
|------|---------|---------------------|---------------|-------|-------|---------|
| Gefitinib | EGFR | recurrent glioblastoma | II | completed | NCT00250887 |
| Gefitinib | EGFR | GBM | II | completed | NCT00014170 |
| Gefitinib | EGFR | GBM | II | completed | NCT00016991 |
| Gefitinib | EGFR | brain and central nervous system tumors | II | completed | NCT00025675 |
| Gefitinib | EGFR | radiation | GBM | I/II | completed | NCT00052208 |
| Gefitinib | EGFR | radiation | GBM | II | completed | NCT00238797 |
| Gefitinib, Temozolomide | EGFR | brain and central nervous system tumors | I | completed | NCT00027625 |
| Gefitinib, Irinotecan | EGFR, topoisomerase I | refractory solid tumor | I | completed | NCT00132158 |
| Erlotinib | EGFR | GBM | II | completed | NCT00337883 |
| Erlotinib | EGFR | GBM | II | unknown | NCT00054496 |
| Erlotinib | EGFR | GBM and other brain tumors | I/II | ongoing | NCT00045110 |
| Erlotinib | EGFR | GBM | I/II | completed | NCT00301418 |
| Erlotinib | EGFR | radiation | brain/central nervous system tumors | I/II | completed | NCT00124657 |
| Erlotinib | EGFR | cytoreductive surgery | recurrent malignant gliomas | ongoing | NCT01257594 |
| Erlotinib, Temozolomide | EGFR | GBM, gliosarcoma | II | completed | NCT00187486 |
| Erlotinib, Temozolomide | EGFR | radiation | GBM | II | completed | NCT00274833 |
| Erlotinib, Temozolomide | EGFR | radiation | GBM | II | completed | NCT00394949 |
| Erlotinib, Temozolomide, Carmustine | EGFR | glioblastoma, gliosarcoma | II | completed | NCT00086879 |
| Erlotinib, Bevacizumab | EGFR, VEGF | glioblastoma, gliosarcoma | II | completed | NCT00671970 |
| Erlotinib, Bevacizumab, Temozolomide | EGFR, VEGF | radiation | GBM | II | ongoing | NCT00720356 |
| Erlotinib, Sorafenib | EGFR, RAF, VEGF | GBM | II | completed | NCT00445588 |
| Erlotinib, Dasatinib | EGFR, SRC | GBM | I | completed | NCT00609999 |
| Dacomitinib | EGFR | recurrent glioblastoma | II | ongoing | NCT01520870 |
| Afatinib | EGFR | refractory solid tumors | II | completed | NCT00875433 |
| AEE788 | EGFR | GBM | I/II | completed | NCT00116376 |
| Lapatinib | EGFR | GBM | I/II | completed | NCT00099060 |
| Lapatinib | EGFR | malignant brain tumors | II | completed | NCT00107003 |
| Nimotuzumab | EGFR | GBM | completed | NCT00561873 |
| Nimotuzumab, Temozolomide | EGFR | radiation | GBM | III | completed | NCT00753246 |
| EGFR Bi-armed Autologous T cells | EGFR, CD3 | glioblastoma, gliosarcoma recurrent neoplasm | I/II | not yet recruiting | NCT02521090 |
| AMG 595 | EGFR | GBM | I | ongoing | NCT01475006 |
| Sym004 | EGFR | recurrent glioblastoma | II | ongoing | NCT02540161 |
| Cetuximab, Temozolomide | EGFR | radiation | GBM | I/II | unknown | NCT00311857 |
EGFR inhibitors

EGFR alteration, including overexpression or gene amplification, is the most frequent form of genetic mutation, occurred in 40-50% of glioblastomas [36, 37]. Logically, EGFR is a promising target for the treatment of GBM. Though promising results was shown in preclinical data, targeting EGFR in clinical trials revealed marginal effects. An overview of ongoing clinical trial in GBM is summarized in Table 1. Information about clinical trials has been retrieved from www.clinicaltrials.gov.

In the clinical trial NCT00250887, the effectiveness of EGFR tyrosine kinase inhibitor Gefitinib was tested in recurrent glioblastoma. Though EGFR was successfully -dephosphorylated, the downstream target remains constitutively active. Therefore the effectiveness was unsatisfactory [38]. Erlotinib, another selective EGFR inhibitor, also showed minimal effect to treat the recurrent glioblastoma (NCT00086879) [39]. Other than single agent treatment, combination therapy was also explored. When Erlotinib was combined with TMZ and radiotherapy in a phase I/II trial, no sign of benefit was showed compared with TMZ controls (NCT00039494) [40, 41]. Additionally, the combination therapy of Erlotinib with VEGF antibody also shows no obvious survival benefit [42]. The failure of targeting EGFR is generally due to the hyperactivation of downstream PI3K/Akt signaling, so the downstream components represents an attractive target for the treatment of malignant brain tumors.

PI3K inhibitors

Currently, the PI3K inhibitors as a single agent or combined with other therapies are being tested in a number of clinical trials (Table 2) [43]. There are pan-PI3K inhibitors and isoform specific PI3K inhibitors [14]. The first generation of pan-PI3K inhibitors is represented by wortmannin and LY294002 [44, 45]. They have showed anti-cancer effect in vivo and in vitro [46-49]. However, both drugs were halted at preclinical studies due to the toxicity, poor pharmacodynamics and selectivity. A new generation of PI3K inhibitors, BKM120 and PX-866, exhibit better drug properties such as high stability and low side effects [50, 51]. BKM120 has anti-proliferative and pro-apoptotic activity in a number of tumor cell lines, human tumor xenograft models and cancer patients bearing PI3K activating mutations [52]. BKM120 was smoothly passed phase I clinical trial and now is undergoing phase II trial among patients with recurrent glioblastoma and activated PI3K pathway (NCT01339052) (Table 2) [50]. At present, BKM120 is also undergoing several clinical trials in combination with radiation (NCT01473901), anti-VEGF monoclonal antibody Bevacizumab (NCT01349660), LDE225 (NCT01576666) and INC280 (NCT01870726) [53]. PX-866 could bind with the catalytic domain of ATP and it acts as an irreversible inhibitor. Though PX-866 could increase median survival time of the animals and show significant anti-tumor activity in GBM xenograft models [54, 55], the recent completed clinical study showed the overall response rate was low (NCT01259869) [56].

| Drug                  | Targets                                      | Partner                                      | Patient group            | Phase | State  | Trail ID |
|-----------------------|----------------------------------------------|----------------------------------------------|--------------------------|-------|--------|----------|
| BKM120, Temozolomide  | Pan-PI3K                                     | surgery                                      | recurrent glioblastoma   | II    | ongoing| NCT01339052 |
|                       |                                              | Bevacizumab                                  | relapsed/refractory GBM  | I/II  | recruiting | NCT01349660 |
|                       |                                              | LDE225                                      | advanced solid tumor     | I     | completed | NCT01576666 |
|                       |                                              | INC280                                      | recurrent glioblastoma   | I/II  | recruiting | NCT01870726 |
| PX-866                | Pan-PI3K                                     | radiation                                   | glioblastoma             | I     | ongoing | NCT01473901 |

Clinical data related to the EGFR was searched until Nov, 2015.
| Drug                        | Targets       | Combination Partner | Patient group                | Phase | State       | Trail ID           |
|----------------------------|---------------|---------------------|------------------------------|-------|-------------|--------------------|
| Sirolimus                  | mORC1         | vaccine therapy     | GBM                          | I/II  | completed   | NCT00047073       |
| Sirolimus, Erlotinib       | mTORC1 + EGFR | glioblastoma        | malignant glioma             | II    | completed   | NCT00672243       |
| Sirolimus, Vandetanib      | mTORC1 + VEGF | glioblastoma        | I/II                         | completed | NCT00509431   |
| Everolimus, Temozolomide   | mTORC1 + radiation | GBM                          | I/II                         | ongoing | NCT01062399   |
| Everolimus, Gefitinib      | mTORC1 + EGFR | progressive GBM     | I/II                         | completed | NCT0085566   |
| Everolimus, Gleevec, Hydroxyurea | mTORC1, PDGFR, BCR-Abl | Cancer                     | I/II                         | unknown | NCT01508104   |
| Everolimus, Temozolomide Bevacizumab | mTORC1, VEGF | radiation           | GBM                          | II    | completed   | NCT00805961       |
| Everolimus, AEE788         | mTORC1, EGFR, VEGFR | GBM                          | I/II                         | completed | NCT00107237   |
| Everolimus, BE235          | mTOR, PI3K/mTOR | radiation           | GBM                          | I/II  | ongoing     | NCT01654349       |
| Temsirolimus, Sorafenib    | mTORC1, RAF, mTORC1 | recurrent high-grade gliomas | I/II                         | recruiting | NCT01434602 |
| Temsirolimus, Doxorubicin  | mTORC1        | resistant solid malignancies | I                           | completed | NCT00703170   |
| Temsirolimus, Docetaxel    | mTORC1        | resistant solid malignancies | I                           | completed | NCT00703625   |
| Temsirolimus, Temozolomide, | mTORC1, radiation | GBM                          | I/II                         | completed | NCT00316849   |
| Temsirolimus, Sorafenib, Erlotinib, Tipifarnib | mTORC1 + EGFR | recurrent GBM or gliosarcoma | I/II                         | completed | NCT00335764   |
| Temsirolimus, Erlotinib, Tipifarnib | mTORC1 + EGFR | recurrent malignant glioma | I/II                         | completed | NCT00112736   |
| Temsirolimus, Perifosine   | mTORC1, Akt, +EGFR | malignant gliomas | I/II                         | ongoing | NCT01051557   |
| Temsirolimus, Bevacizumab  | mTORC1 + VEGF | radiation           | GBM                          | II    | completed   | NCT00800917       |
| Ridaforolimus              | mTOR          | Glioma              | I/II                         | completed | NCT0087451   |
| CC-115                     | DNA-PK/mTOR   | advanced solid tumor | I/II                         | ongoing | NCT01353625   |
| CC-223                     | dual mTOR inhibitor | surgery, supportive care | advanced solid tumor | I/II                         | NCT01177397       |
| XL765, Temozolomide        | dual PI3K/mTOR | radiation           | GBM                          | I     | completed   | NCT00704080       |
Akt inhibitors

Akt is a central player in the EGFR/PI3K signaling pathways. Evidence shows that Akt play an important role in tumor proliferation and radiosensitivity [57]. One of the most promising Akt inhibitor, perifosine, inhibits Akt activity by preventing its translocation to the cell membrane [58, 59]. Currently, perifosine is being clinically tested in a number of different cancers [60, 61]. Perifosine has several drawbacks such as limited ability to penetrate blood-brain-barrier (BBB) and gastrointestinal side effects. A phase II trial of perifosine in recurrent GBM was ongoing but only marginal effect was shown (NCT00590954).

mTOR inhibitors

As downstream targets of phosphorylated Akt, inhibition of mTOR would also be another therapeutic approach to reduce the effects of constitutively activate Akt in GBM. mTORC1 inhibitors mainly contain rapamycin (sirolimus) and its analogues, such as RAD001 (everolimus), CCL-779 (temsirolimus) and AP23573 (ridaforolimus) [62]. Rapamycin inactivate mTORC1 through altering the conformation of the kinase. Though rapamycin and its analogues exhibit efficacy of mTOR inhibitors in both in vitro and in vivo models [63, 64], they would arose hyperactivation of Akt and mTORC2 by some feedback loop and pathway crosstalk [65]. Rapamycin shows anti-tumor activity in a phase I trial for patients with recurrent PTEN-deficient glioblastoma (NCT00047073) [66]. Unfortunately, phase II clinical trials for rapamycin analogs fail to achieve promising results (NCT00515086, NCT00016328, NCT00022724, and NCT00087451) [67-71]. The limited efficacy might result from the feedback loops and crosstalk with other pathways. Recently, more exploration was focusing on the combination treatment of rapamycin analogs with other modalities [71]. The combination of EGFR inhibitor erlotinib with sirolimus or temsirolimus was tested in clinical trials (NCT00112736 and NCT0062243). However, either of trial shows promising results [72, 73]. A Phase II study of everolimus with bevacizumab as part of first-line modality therapy for glioblastoma was feasible and efficacious (NCT00805961) [74], further studies are still need. As combined inhibition of Akt and mTOR by perfosine and temsirolimus inhibited murine glioblastoma growth no matter PTEN status, a phase I/II trial in recurrent high-grade gliomais ongoing (NCT01051557) [75, 76]. Metformin is a widely prescribed anti-diabetic drug and many studies indicate that metformin inhibits cancer proliferation through the inhibition of mTOR [77]. The efficacy of metformin on glioblastoma was tested in clinical trial NCT01430351 and NCT02149459. In NCT02149459, metformin was combined with radiotherapy. In NCT01430351, metformin was combined with TMZ. Both of the trials are still in phase I state. Getting specifically mTORC2 could thereby be a better approach, since it would directly block Akt phosphorylation without perturbing the mTORC1-dependent feedback loops [78, 79]. In contrast to mTORC1, mTORC1/2 inhibitors can restrain Akt phosphorylation at Ser473, thus also inhibit mTORC2 at the same time [63]. AZD8055 is a potent small molecular ATP-competitive inhibitor. In vivo, AZD-8055 reduced S6 and Akt phosphorylation thereby leading to the reduction of tumor growth [80]. It is implicated that AZD8055 may provide a more promising therapeutic strategy than rapamycin and analogues [81]. Currently AZD8055 has completed the phase I clinical trials (NCT01316809).

PI3K/mTOR dual inhibitor

Since mTORC1 inhibitors could induce the loss of feedback inhibition of PI3K activation, drugs targeting PI3K and mTOR kinase simultaneously thereby become a superior option [82]. Active site ATP-competitor is a class of dual PI3K-mTOR inhibitor, which structurally

| Drug Name       | Target    | Disease                  | Status       | Clinical Trial ID   |
|-----------------|-----------|--------------------------|--------------|---------------------|
| XL147, XL765    | PI3K, PI3K/ mTOR | glioblastoma, astrocytoma, Grade IV | I completed | NCT01240460         |
| PKI-587         | PI3K, class IA, mTORC1/C2 | solid tumor | I completed | NCT00940498         |
| AZD8055         | mTOR      | GBM                      | I completed | NCT01316809         |
| INK128          | mTORC1/2  | Bevacizumab               | I recruiting | NCT02142803         |
| Pembrolizumab, Pictilisib, NVP-BEZ235, Ipatasertib | PI3Ka/δ, PI3K/mTOR, Akt1/2/3 | Glioblastoma | I/II                | NCT02430363         |
| Metformin       | mTOR      | GBM                      | I ongoing    | NCT01430351         |
| Metformin       | mTOR      | radiation                | I recruiting | NCT02149459         |

Clinical data related to the EGFR was searched until Nov, 2015.
targets the kinase domains of both PI3K and mTOR. PI-103 was the first dual mTOR/PI3K inhibitors that inhibited mTOR in an ATP-competitive manner [83]. In vivo study showed that PI-103 led to G0-G1 cell cycle arrest thereby inhibiting the proliferation and invasion of tumor cells [84]. However, PI-103 was halted in the preclinical period due to the poor pharmacokinetic properties. NVP-BEZ235 is a promising PI3K/mTOR dual inhibitor exhibiting improved anti-tumor potential compared to rapamycin analogs [85-88]. In preclinical test, study demonstrated that NVP-BEZ235 significantly prolonged the survival of tumor bearing animals without eliciting obvious toxicity [89]. Therefore, NVP-BEZ235 has entered phase I and phase II clinical trials with everolimus in patients with malignant solid tumors (NCT01508104). Other dual PI3K and mTOR inhibitors, such as PKI-587 and XL-765, have shown favorable activity in preclinical settings. XL-765 has completed the trial in combination with radiotherapy and TMZ for GBM as well as in subjects with recurrent GBM (NCT00704080). PKI-587 and XL-765 have recently completed the phase I clinical trials for the treatment of solid tumors (NCT00940498) and recurrent GBM who are candidates for surgical resection (NCT01240460).

THE LIMITED FACTORS OF TARGETED THERAPY BASED ON PI3K SIGNALING PATHWAY

Though more and more PI3K/Akt/mTOR targeted drugs emerge, they are still undergoing preclinical or clinical trials. Targeted therapy for GBM has yet to demonstrate an appreciable clinical survival benefit. At present, here are some possible reasons for the limited therapy effect: (1) Blood Brain Barrier. It’s the most likely explanation for why targeted drugs cannot reach effective concentrations (2) Heterogeneity of GBM. No doubt the outcome of drug efficacy is much influenced by the genetic background of the tumor. In malignant tumors, molecular phenotype of the same tumor in different location may totally diverse and molecular phenotype of the same tumor in different people may also vary. Thereby the sensitivity to targeted therapy may vary. (3) The activation of alternative pathways leads to immune escape. In clinical trials, only a small proportion of the clinical trials in malignant gliomas concurrently conducted pharmacokinetic studies and most of these studies collect blood samples to work out plasma clearance, rather than directly analysis of cerebrospinal fluid or drug concentration in tumor tissues. Collectively, there are all relevant restrictions to targeted therapy based on PI3K signal pathway.

CONCLUSION AND FUTURE PROSPECTS

As we have discussed here, PI3K/Akt/mTOR signal pathway after activation of EGFR is one of the most significant signal pathways in tumor cells. It has confirmed that it plays an important role in the genesis and development of glioma. At the moment, targeted therapy towards intracellular signal pathways has not achieved satisfactory result yet. A future perspective for GBM therapy is combination of multiple targets and personalized treatment. Although issues like cross-talk signal pathways or tumor heterogeneity tarnished the efficacy of therapy targeted PI3K/Akt/mTOR as we expected, we still believe that it will light up a new way in glioblastoma therapy. Recent study showed that targeting HSP90 and histone deacetylases could enhance the therapeutic effect of TMZ combined with radiotherapy [90].

Abbreviations

GBM: glioblastoma multiform, PI3K: phosphatidylinositol 3-kinase, mTOR: rapamycin-sensitive mTOR-complex, EGFR: epidermal growth factor receptor, TMZ: temozolomide, RTKs: receptor tyrosine kinases, PTEN: phosphatase and tensin homolog deleted on chromosome ten, pS6k: ribosomal protein S6 kinase, eIF4E: eukaryotic initiation factor 4E, 4EBP1: eukaryotic initiation factor binding protein 1, PIP2: phosphatidylinositol-3, 4-bisphosphate, PIP3: phosphatidylinositol-3, 4, 5-bisphosphate, BBB: blood-brain-barrier, PDK1/2: phosphoinositide-dependent kinase 1/2

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CONFLICTS OF INTEREST

There is no conflict of interest.

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