

### Example 1

**3.A Significance:** Diabetes patients are at high-risk for cardiovascular disease (CVD) (Malik , Lopez, Chen et al, 2007; Saydah, Fradkin, and Cowie 2004; Buse JB et al 2007), the leading cause of death, disability, and health care expenditure in the US (CDC 2007). While randomized control trials have repeatedly shown the effectiveness of medications to control hypertension, hyperlipidemia, and hyperglycemia in reducing CVD-related death and complications (ADA 2003; Snow, Aronson, Hornbake et al 2004; Snow, Weiss, Mottur-Pilson 2003), these medications are often underutilized (Shrank, Asch, Adams et al 2006). Studies suggest only 50-70% of patients with diabetes achieve good adherence to their medications (DiMatteo 2004; Feldman, Bacher, Campbell et al 1998; Avorn, Monette, Lacour 1998), and up to half of chronic illness patients prescribed medication discontinue use within a few years (get cite for this). This non-adherence to CVD risk factor medications in patients with diabetes is associated with increased rates of hospitalization and death (Ho, Rumsfeld, Masoudi et al 2006; Laur and Nau 2004; Sokol, McGuigan, Verbrugge et al 2005).

Lack of timely access to medication refills can be a significant barrier to achieving good medication adherence (Duru, Gerzoff, Selby et al 2009). Mail order pharmacies, which can streamline the process of access to medications and reduce the need to travel to medical facilities, can potentially improve access to needed medications for patients with chronic illness (Duru, Schmittiel, Dyer et al 2009). Recent research suggests that mail order pharmacy use is associated with greater adherence to medications in patients with diabetes and with greater improvements in LDL control in new users of statin medications (Schmittiel, Duru, Dyer et al under review).

While mail order pharmacies are widely used in the U.S. – one recent estimate suggests that up to 1/3 of medications for chronic illness are delivered to patients via the mail (Federal Trade Commission 2005)– the impact of mail order pharmacy on patient safety and use of preventive health services is largely unexplored. For example, mail order pharmacy may improve clinical outcomes through increased access to CVD risk factor medications, but it may also serve as a barrier to access to pharmacy consultation and other services designed to prevent adverse events (Duru, Schmittiel, Dyer et al 2009). The proposed research will contribute to our knowledge of the effects of mail order pharmacy by assessing whether mail order use is associated with improved patient safety, appropriate services utilization, and clinical outcomes in diabetes patients. *This contribution will be significant because understanding the effects of mail order pharmacy use on patient safety and clinical outcomes will help health systems design safe and efficient medication delivery systems with the potential to improve CVD-related outcomes in diabetes patients.* Improved medication adherence through mail order pharmacy use has the potential to decrease CVD-related hospitalizations and deaths in diabetes patients. Through a full assessment of the risks and benefits of mail order pharmacy use, we can gain important knowledge on how to potentially improve and expand the use of mail order pharmacy services.

**3.B Innovation:** Most interventions designed to improve medication adherence focus on changing patient or provider behaviors (McDonald, Garg, Haynes 2003; Haynes, Ackloo, Sahota et al 2007). These interventions tend to be both intensive and costly, and few have been shown to be effective (Lee, Grace, Taylor 2006; Murray, Young, Hoke et al 2007; McDonald, Garg, Hayes 2003). While the World Health Organization has advocated for considering the impact of healthcare system and other ‘structural’ factors on medication adherence, system-level barriers to medication use are rarely addressed by clinicians or healthcare researchers (Sabate 2003). *The proposed research is innovative because it addresses the potential of a healthcare system-level structure – in this case, the provision of mail order pharmacy service – to improve patient outcomes.* Many researchers and policy makers have lamented that the field of medication adherence research is “stuck” with few good options for moving forward (Vermeire, Wens, Van Casteren et al 2001; van Dulmen, Sluijs, van Dijk et al 2007). Research that comprehensively assesses the impact of system-level interventions such as mail order pharmacies can significantly advance the field of structural interventions to improve patient health.

## Example 2

**11.A SIGNIFICANCE:** PCR detection of *EWSR1* fusion transcripts is currently the only available method for detecting occult bone marrow Ewing sarcoma cells. This technique has several important disadvantages that limit its utility. This approach requires prior knowledge of the type of *EWSR1* translocation present in the tumor. The type of translocation is not known at initial diagnosis and may not be ascertained in all patients. If only the most common translocations are evaluated, transcripts reflecting less common translocations will be missed. Additional false negative results can be obtained because mRNA is sensitive to degradation. PCR has not been used to quantify the number of *EWSR1* transcripts and the tumor cells themselves cannot be further characterized by PCR. With these limitations in mind, we developed a novel flow cytometry approach for detecting occult Ewing sarcoma cells. While PCR-based studies have detected occult bone marrow Ewing sarcoma cells in approximately 25% of patients, our preliminary studies indicate that flow cytometry detects these cells in 90% of patients, though the levels vary among patients. The proposed research will utilize this new technique to evaluate the prognostic impact of extent of bone marrow micrometastatic disease.

The approximately 25% of patients with Ewing sarcoma who have detectable bone marrow fusion transcript by PCR appear to have an inferior outcome.<sup>3,6</sup> Persistence of bone marrow fusion transcript despite treatment with chemotherapy also confers an adverse prognosis.<sup>2</sup> These studies have focused only on the presence or absence of occult bone marrow involvement and have not evaluated the prognostic impact of the extent of bone marrow disease. Given the limitations of PCR, this marker has not been used to modify therapy for patients with Ewing sarcoma. Instead, patients with localized Ewing sarcoma receive standard therapy that ignores the potential prognostic impact of bone marrow micrometastatic disease at diagnosis or in response to therapy. In childhood acute lymphoblastic leukemia, evaluation of minimal residual disease by flow cytometry has become one of the most powerful prognostic factors.<sup>15</sup> This marker is now routinely used to modify therapy for leukemia patients based on their response to initial therapy. *The proposed research has the potential to improve outcomes for patients with Ewing sarcoma by enabling better risk-stratified and response-based therapy.*

While Ewing sarcoma is a relatively uncommon malignancy, the central hypothesis that flow cytometric detection of bone marrow micrometastatic disease can aid in risk stratification may ultimately be applied to other patients with sarcoma. The flow cytometry assay developed in the proposed research can be adapted for evaluation in other sarcoma histologies. More precise risk stratification is of particular importance in sarcoma due to the wide variety of histologic subtypes that are often treated in a uniform manner.

**11.B INNOVATION:** Given the aforementioned limitations of PCR for the detection of bone marrow micrometastatic disease in Ewing sarcoma, we sought a new approach. The available methods used to detect disseminated tumor cells in patients with carcinoma typically rely on the presence of carcinoma-specific markers.<sup>16,17</sup> These methods cannot be applied to patients with Ewing sarcoma or other sarcoma histologies because these mesenchymal malignancies do not typically express these markers. We therefore developed a new assay specifically for Ewing sarcoma. While we have focused on Ewing sarcoma, our flow cytometry approach provides a model that other investigators can apply to additional sarcoma histologic subtypes and more broadly to other malignancies that do not routinely express carcinoma markers.

This novel approach takes advantage of the immunophenotype of Ewing sarcoma cells. These cells show nearly universal membrane CD99 expression.<sup>18,19</sup> Unlike early T-cells and monocytes that also express CD99, Ewing sarcoma cells do not typically express CD45, the leukocyte common antigen.<sup>9,10,20,21</sup> We have performed a series of preclinical studies to determine the ability of flow cytometry to detect Ewing sarcoma amidst a background of hematologic cells. These recently published studies demonstrate that this technique can reliably identify small quantities Ewing sarcoma cells in blood and bone marrow.<sup>22</sup>

This technique appears to be better suited than PCR for the detection of occult Ewing sarcoma cells as part of a risk-stratified and response-based clinical trial. Since CD99 is nearly universally expressed on these cells, this assay can be applied to patients with Ewing sarcoma regardless of the type of *EWSR1* translocation. The technique avoids the issue of mRNA degradation and quantitative results are available in real time. The flow cytometry foundation of this assay will also allow us to study the biology of these cancer cells using more advanced techniques. These techniques include phospho-flow cytometry to interrogate specific signaling pathways and cell sorting to collect the micrometastatic cells for further study. *In addition to the innovation inherent in our newly-developed technique, the technique also provides an important new tool that can be applied to clinical and biological studies of Ewing sarcoma.*