Discuss the role histopathology now has, or could have in the future, in the field of Pharmacodiagnostics, targeted therapies/personalised medical treatment

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Introduction:

Pharmadiagnostics is the highest extension forward enhance patients' cases with tumour disease which has started at the beginning of 21th century (Kierkegaard and Bartlett, 2008). Basically, it can connect between cure results such as response and resistance. It is wildly used test in genetics associations researches which has been identified in high response to the drugs therapy for each individual either they will benefits from these treatment or not (Nadji and Nassiri, 2008; Bartlett., 2005). It is estimated that pharmacodiagnostics have used as ideal technique in oncology to improve and enhance targets therapies (Jorgensen et al., 2012). The need for these combinations of personalized treatment is to certain the exact targets and measure the activity and response for this target (Bartlett, 2005; Kierkegaard and Bartlett, 2008). The target therapy was used during 1972 significantly as cancer cure because these drugs are included in the diseases procedures of tumour by using particular molecular (Plevin et al., 2010). It has been recognized that applying HER2 (ERBB2) assay for detecting tratuzumab in breast cancer is one of fundamental discoveries in pharmacodiagnostics test (Bartlett, 2005). In modern studies Pharnacodiagnostics is more preferable than chemotherapy as a result to using drugs based on the human genome which related to molecules markers, while in chemotherapy the cells might be destroyed completely. The aim behind identifying these molecules unique will help to recognize cancer types and then the targets therapy can be detect as well (Nadji and Nassiri, 2008). Moreover, many researchers are concentrated on drugs to fit all the patients' cancer while the fact that different people have different agents (Kierkegaard and Bartlett, 2008). This paper will shed light to the most important roles for applying histopathology in several cancer examples. Attention will be made forwards the ideal techniques which have been applied for target diagnosis as well as therapies will be discussed in details. At the end of this paper some of future plans will be drawing in this particular pathway.

1. Therapy targets in breast cancer:

Mainly, there are two types of targets can be distinguish in cancer treatment. Firstly, proteins are basically enzymes found in the cells transductions such as protein tyrosine kinase (TKs) which has used considerably in tumour cure because their abnormal efficacy will lead to detect cancer stages. Another example can describe is (EGFR) and (HER2) which able to inhibit the signalling pathways. Furthermore, targeted therapies (VEGFR) receptor which plays an essential roles in genetics factors. Secondly, group of drugs can be successfully used in anticancer which is monoclonal antibodies such as tratuzumab one of these drugs which target (HER2), also, cetuximab which target (EGFR) and bevacizumab which stop the efficacy to (VEGFR). Another target type is molecule unique to block the unnatural activity of tumour. For example, TK inhibitor which involved imatinib. Using these groups of drugs consider important because their benefits, cheap and simple to use orally (Nadji and Nassiri, 2008).

2. The roles of histopathology in breast cancer:

Breast cancer has been classified as the highest incidence disseises in women which threaten their life and the second cause for morbidity (Stopeck et al., 2012). In addition, this cancer forms around 32% among another cancers in women (Kumar and Badve, 2008). In fact, this disease still not clearly understood because genetics reasons or environmental associated causes. Medical oncologists have focused on targeted therapy, personal therapy in order to reduce the cases rates. This cancer can be distinguish as model disease to explore targeted therapy. The usage of several agents such as estrogen receptor (ER), progesterone receptor (PR) and finally the human epidermal growth factor receptor (HER2) as active drugs have expanded the chance to cure breast cancer. In spite, of these discoveries there are still some challenges which form by different methods to use these targets and resistance of some drugs. All of these receptors play a crucial pathway in breast cancer.

3. PR receptor and ER receptor:

Progesterone receptor is a hormone steroid which includes antibody to identify the expression level of this receptor in breast cancer tissue. In case of not finding PR the patients will not survive and will happen frequently (Allerd, 2006). The presence of antibodies in PR trigger to high percentage of recognizing the disease. Illustration for staining ER and PR by using IHC and clarify the positive ER around 90% while PR 10% (figure1).

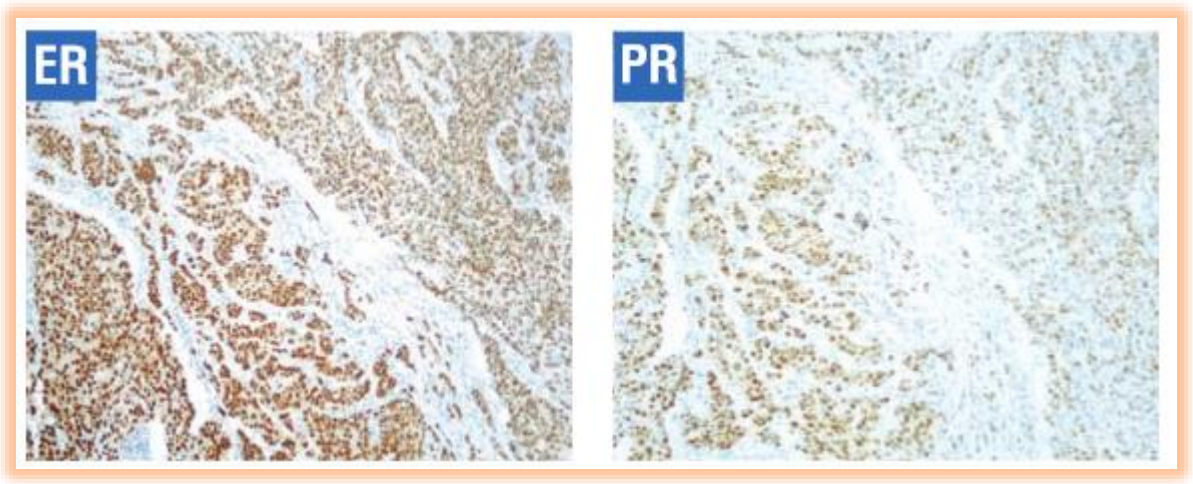

Figure1. the different IHC staining between ER and PR in IHC (Allerd,2006).

Estrogene receptor forms an important role in human breast cancer as it linked with untreated target. It is recommended that the analysis to both of these receptors should be done to all the breast cancer. After identifying them in patients, the results should detect the expression to both of them which lead to response to endocrine drugs. This happens because breast cancer has some receptors which binds ER, PR then will give positive ER, PR growth. The main alteration between these receptors can be identified by histological methods. For example, in case of applying IHC can easily measure ER, PR receptors. Then the findings show that H grade between (0-300) around (0-100% or in numbers as 0,1+,2+,3+. Actually these grades perhaps change from laboratory pathology to another, but the main concept is when grade high this means the receptors in breast cancer high as well(Beikman et al.,2013). In case of HER2 receptor positive breast cancer this will increase in tumour size and reduce it when it happen again also, the patient will not survive and lead to high morbidity (Beikman et al.,2013). As it will mention later several techniques have applied to identify HER2 positivity such as IHC and FISH. As we can see in the following figure the main graded which been evaluated in IHC staining for estrogene receptor (figure2).

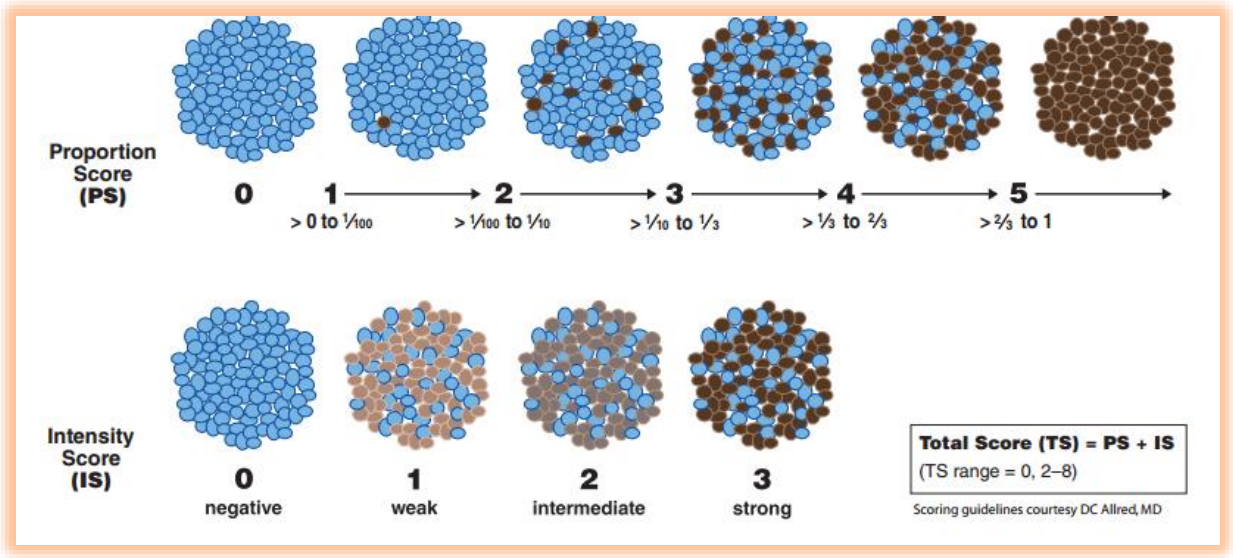

Figure2. the principles of scores for ER in IHC staining (Allerd, 2006).

4. HER2 target:

Human epidermal growth factor receptor1 (EGFR) and HER2 both of them consider the most crucial target genes which have used widely in cancer studies as therapies. Their expressions is abnormal that is because has mutation connect with breast cancer. This leads at the end to high survive from cancer (Tani, Hatanaka and Hashizume, 2006). In addition, HER2 can produce 15-20% proteins which use easily as target therapy and his membrane record as 0, 1+, 2+, 3+(Kumar and Badve.,2008). As it recorded in IHC assay that 0-3+ detect on the amount of HER2 receptor protein, while, 0-1+ refers to HER- negative and 2+ called borderline. Finally, the test results show score3+ means HER2 +positive (Caplazzi, 2009). It is evaluated that by these scores can identify the exactly standard of cases. A study conducted by Beikman et al showed that the percentage of cancer was 65-85% in this study. Also, the grades of cancer were 1, 2, 0.9 and 1.0 cm refer to TI estrogene receptor positive with H 10 grade. Finally, HER2 was unclear 2+ by using IHC test with FISH as well. In order to identify the case molecularly it is suggested to clarify estrogen receptor ER, PR and HER2 each of them individually. Some researchers suggest that the main target therapy are depends on ER,PR,HER2 cases and this will occur after finishing lab test for blood count and liver functions. Further drugs need to apply as supportive such as trastuzumab which uses in case of positive result for more treatment based on chemotherapy or endocrine. Particularly, in this case study was examined trastuzumab was preferable drug because in IHC and FISH assays the HER2 was unclear and his grade was only 5. The latter indicates the overexpression is protein HER2. Therefore, women with ER receptor positive need to have damoxifen instead of aromatase (Beikman et al., 2013). According to the Breast Cancer Organization, 2013 there are three drugs can work as anti HER2 breast cancer such as, trastuzumab, lapatinib and pertuzumab. In the next picture we can notice the differences in immunostaining (figure 3).

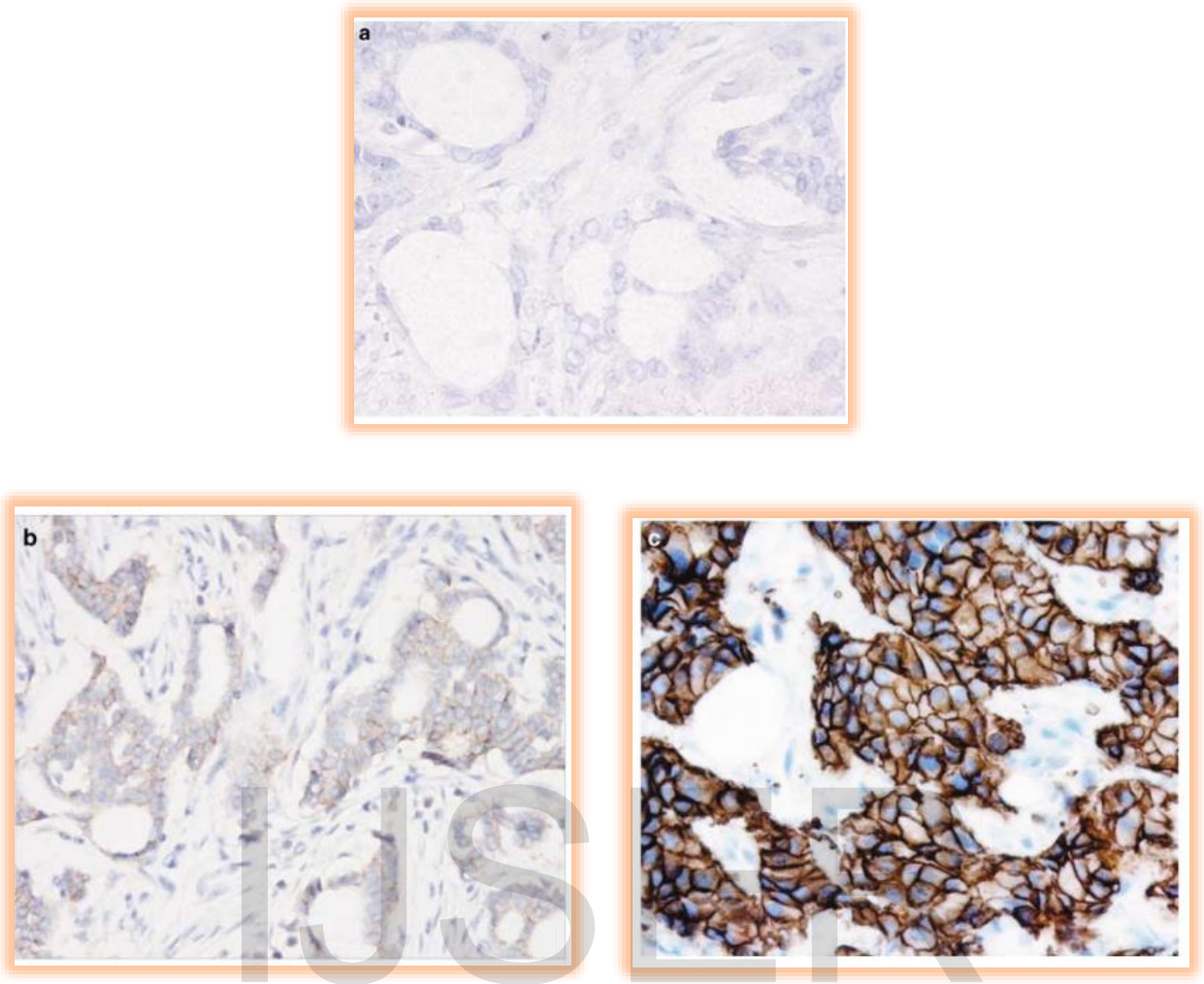

Figure 3. examples HER2 immunostaining, a)0 negative ,b)2+ equivocal and c)3+positive.

(Gown, 2008)

5. Applications of pharmacodiagnostics in cancer:

Although, there are variety of diagnosis methods of cancer, histopathology still form as an ideal manner in cancer diagnosis tissues. These techniques help to detect on the neoplasia and the presence of receptors which will response to drugs target as well as predict some of important information about the disease. **IHC immunohistochemistry** is one of the main molecular novel techniques which applied widely in variety of clinical analysis pathology. Particularly, in the detection of HER2 in breast cancer (Bartlett, 2006). Also, to estimate progesterone and estrogene receptors. It has used significantly to identify antigens in specific cells and differentiated from others because it is more understand, cheaper, less time than another methods. Comparison with FISH test is less sensitive and less accurate (Caplozzi, 2009; Gwan, 2008). Studies indicate that ER is weak because there was 10% response among 280patients. Another study found that PR weak value to predict as well.

That is the reason behind the need for further methods to evaluate prognostics (Allred, 2006). The simple steps will view in next (figure4).

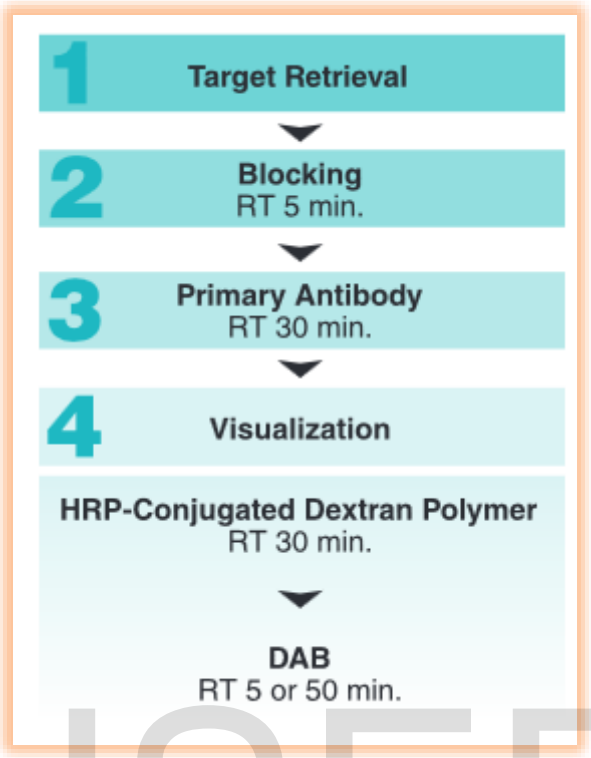

Figure4. IHC staining in simple stages in the lab (Tani, Hatanaka and Hashizume, 2006)

FISH fluorescence in situ hybridization test which is basically identify HER2 gene quantitivity by mixing formalin-paraffin with breast cancer tissues. This multi colure from 2-4+ FISH for gene HER2 in breast cancer (Bartlett, 2006). Some researchers suggest that further drugs should apply as target therapy but after finishing lab test. For example, the reason to use this test because sometimes IHC give unclear results such as breast cancer cells are HER2 + or -. However, using this method is ideal for target diagnosis but it still limited for some types of cancer and only small number of centres have this method. In addition, it requires high expertise for the practical work, expensive, should has fluorescent microscope and spend more time than IHC. In this case, it is difficult to perform this methods in poor countries and this issue will affect economically the country which apply FISH. But it considers more sensitive than IHC as well (Gwon, 2008).The basic steps for FISH can show as bellow:

DNA synthesis → Specimens' collection (Cells + tissues) → Incubation tissue in acid

Decrease agents to break protein → Protease digestion

(Gown, 2008).

Since decade another technique was explored which represents by microarray technology is crucial for detecting gene expression profile and contributing in oncology practices. A study conducted by Colombo et al confirm that microarray methods in ER positive tumours trigger to detect on 165 genes expression with ER. In fact that this technique improve our knowledge about breast cancer biology in both manner, by clinical and research process (Colombo et al., 2011). (figure 5).

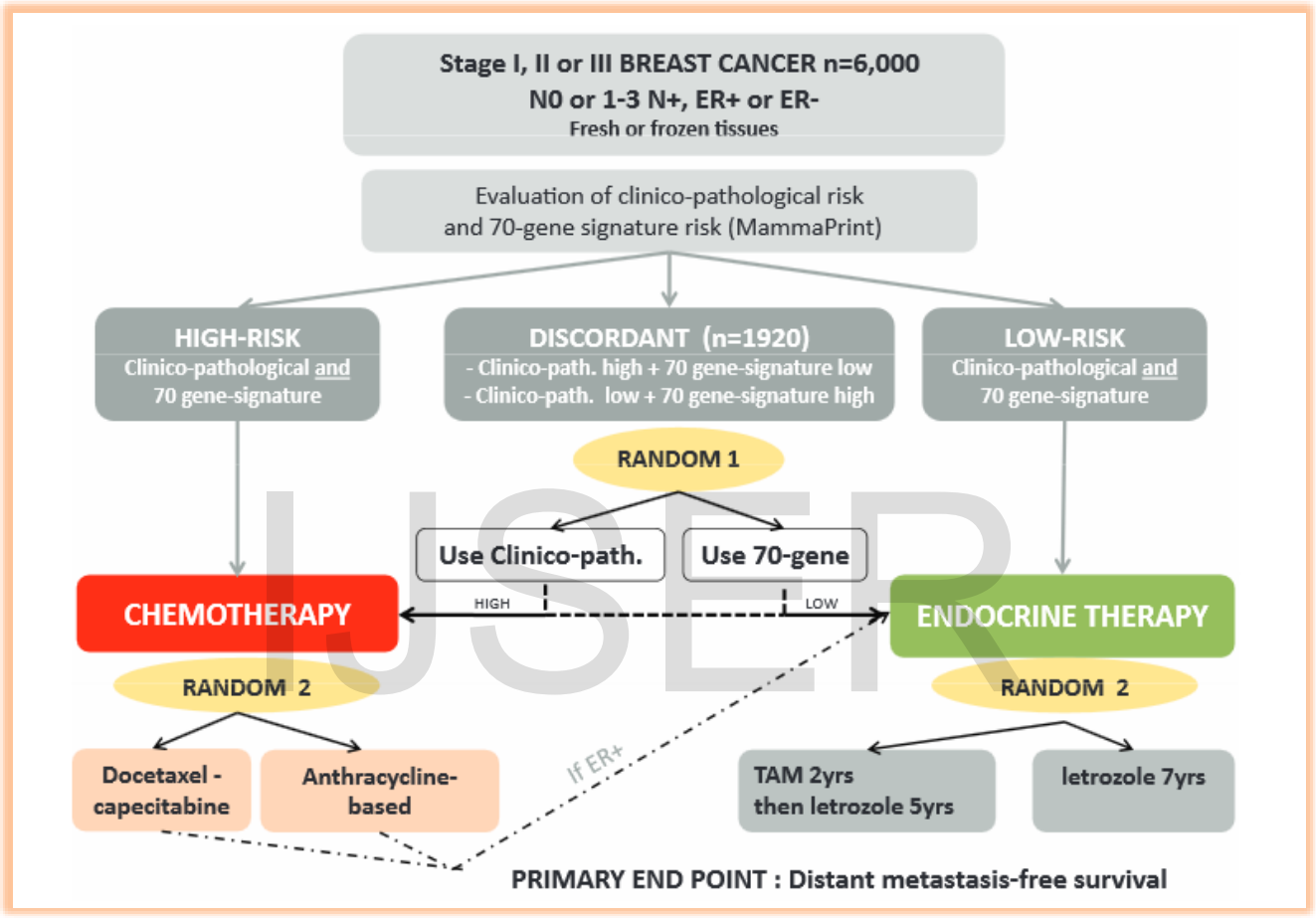

Figure5. Microarray technique (MINDACT) indicating Mamaprint and Oncotype (Colombo et al., 2011).

6. GIST- gastrointestinal stromal tumour

The main role of histopathology in pharmacodiagnostics can form in another example such as GIST, this term refers to one of the rare cancer which find in small rates in digestive system, and some types of this cancer are not harmful. However, it will develop cancer if the case not treated. 60% cases found in the stomach or in any part of the gastrointestinal tract. About 900 cases in the UK which identified every year (WHO, 2013; Joensuu et al., 2013). There are three types of GIST; leiomyomas, leimyosarcomas and schwannomas which are depend on the histological features. In order to diagnose the GIST some methods need to apply such as IHC by using antibodies to investigate whether the protein

is found in tumour specimens or not. Immunostaining technique used to detect GIST cancer by testing the expression of KIT protein or as it known CD117. The latter mutation lead to develop GIST then expressed by gut cells or called cajal (ICC) and another type of cells such as mastcell or melanocytes in the skin. A positive results from kit antibody test refer to GIST diagnosis such as 5% grade + .While the kit negative can be detect by using markers such as A(PDCFRA) receptor and protein kinase (PKC)(Kang, Jung and Hwang.,2010). Further techniques used to diagnose GIST microarray tissue on the patient samples. Also, CT, endoscopy, ultrasound, biopsy and MRI all these tests can be used for further investigation before surgical resection (WHO, 2012). Using light microscopy by pathologists of IHC markers such as anti- CD34 because when we gain on c-kit negative cases, it will be hard to investigate them by these method. Therefore, it should apply another tests because these markers might have the same morphological characteristics as c-kit+. After detecting on c-kit (CD117) which considers very essential to gain on sensitive results of GIST and these kit show positive expression in GIST around 98%. Imatinib mesylate uses as therapy and some of antibodies such as DOG-1, PKC-theta and CD34 with c-kit are used to detect the high level of specificity and sensitivity. The issue with this type of cancer still associated with the diagnosis methods such as IHC because the differences in c-kit sequencing and kit. Studies illustrate that patients which suffer from GIST with 9 and 11 mutants' cancer develop their case better than people with wild genotype (Joensuu et al., 2013).

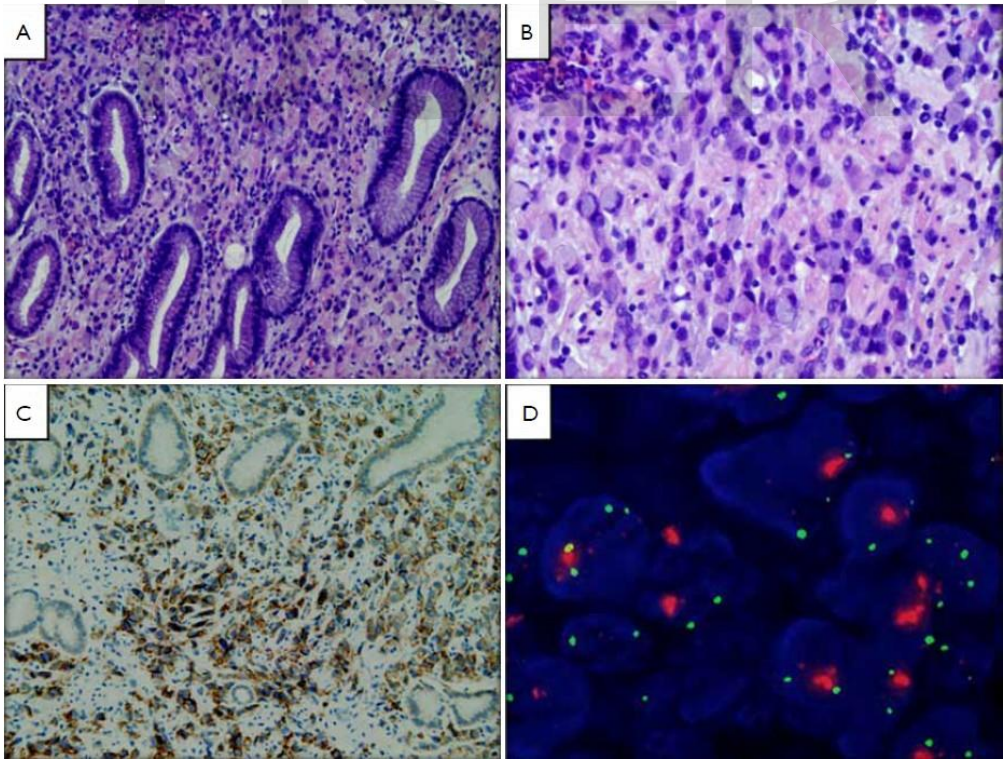

Figure 6. H and E staining for GIST cancer A. low power for tumour cells. B. high power. C. IHC of HER2 +3 tumour. D. FISH shows ring cells HER2 is red signal and CEP17 is green (Ness et al.,2012).

7. Lung cancer:

This type of cancer can be detect by using electrical skin resistance test during 10 min. more recent studies indicate that using low-dose CT scans which basically it is an image of the lungs by applying small part of radiation. Four types of resistance produce by the patients. Non-small cell lung cancer (NSCLC) classifies into different type depends on their histologic characteristics which are adenocarcinoma, squamous cells carcinoma. In the past the treatment was very weak for 2-4 weeks survive time only. Recently, studies have used platinum with gemcitabine, vinorelbine and docetaxal. These drugs lead to high survival results for lung cancer patients. More recently, researchers have applied more specific medicine pemetrexed which produces high activity particularly against non-squamous cell carcinoma. Farther exploration between histologic and molecular approach such as using (EGFR) mutation and ALK gene with target therapy ceritinib which has used more recently for treatment to NSCLC (Rolfo et al., 2015). Another studies indicate that mutation of EGFR which around 90% in exon 21 will rise up the EGFR efficacy then lead to produce cancer and cell reproduction. This cancer can be detected as well by applying IHC with TKIS inhibitor. It is estimated that in order to detect on this type of cancer need to use biopsy specimens. If there is pulmonary adenocarcinomas so this means no need to perform immunohistochemistry. Because the tumour has already investigated. A study carried out by Aisner and Marshall, 2012 that 50%of lung cancer cases in stage IV diagnosis. While, 20% of another cases with CT screening. Histologically, they have proved that EGFR is more likely found in women lung cancer and non-smoker women. They suggest the first line drug with EGFR TKIs are very crucial for lung cancer patients comparing with chemotherapy treatments. A more important findings that HER2 was finding as well in small cases of NSCLC. This receptor as it was discussed previously has mutations which will activate in this case, therefore the inhibition can be identified easily (Asiner and Marshall, 2012). As we can see in (figure7).

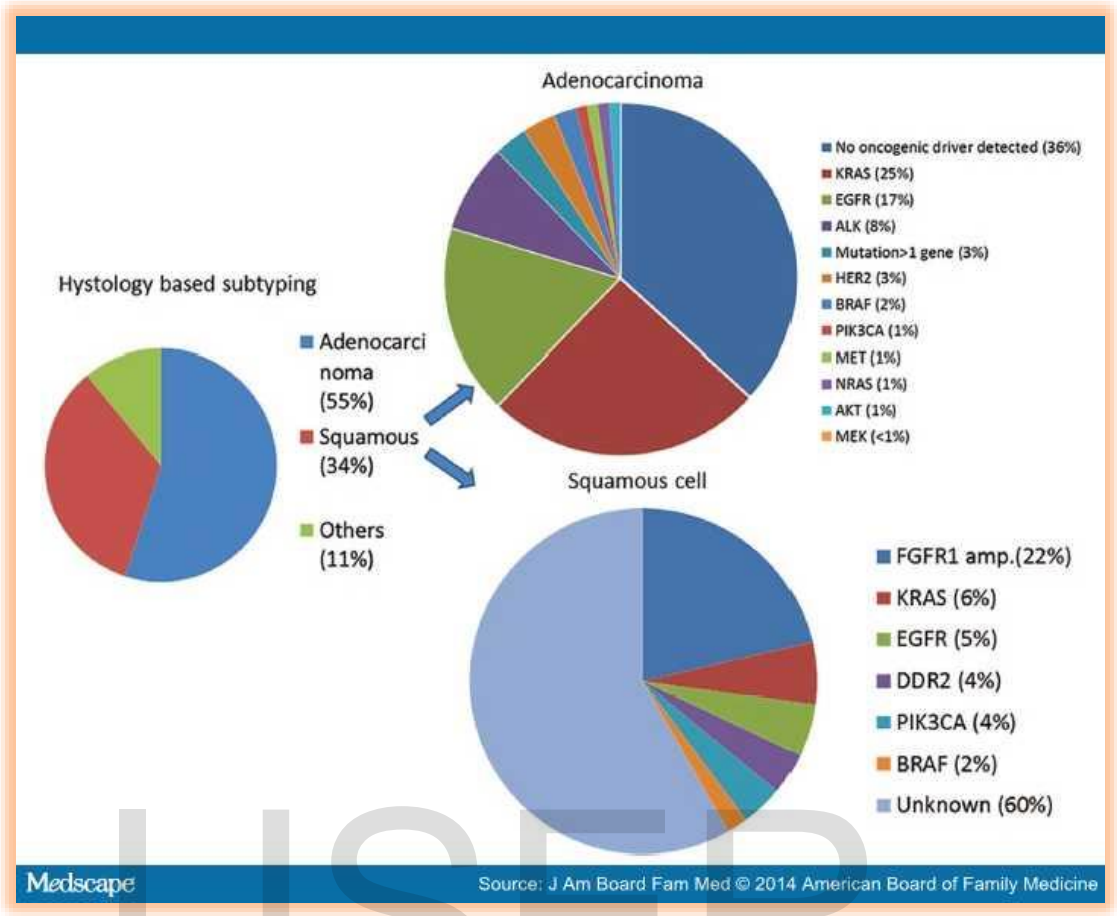

Figure 7. indicate different type in lung cancer with variety of target therapy (Rolfo et al., 2015).

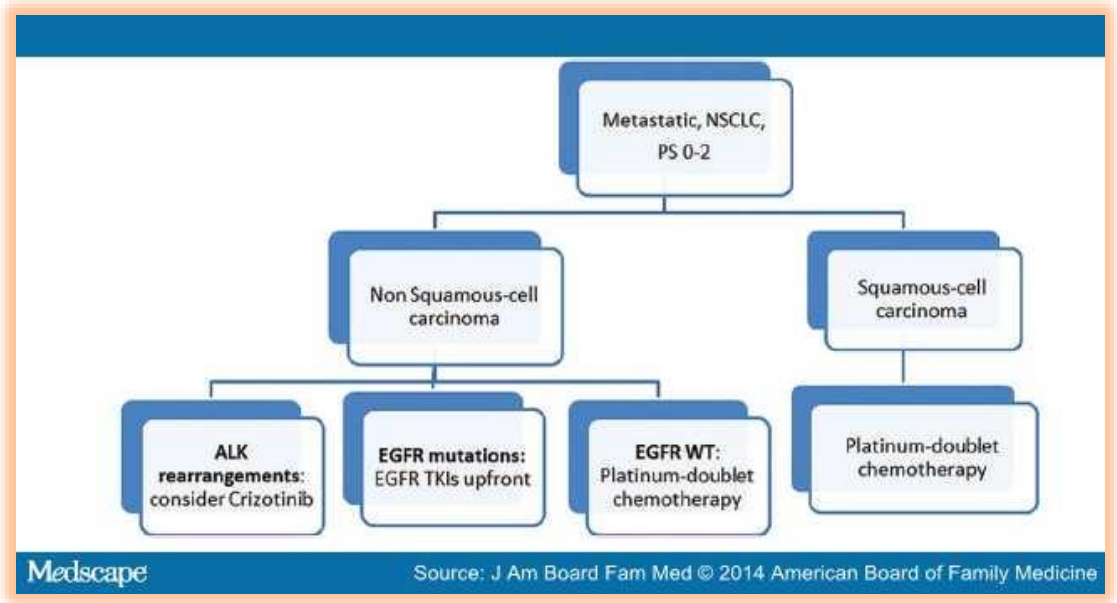

Figure 8. the treatment stages for (NSCLC) by using (EGFR) receptor, ALC and TKI.(Rolfo et al.,2015).

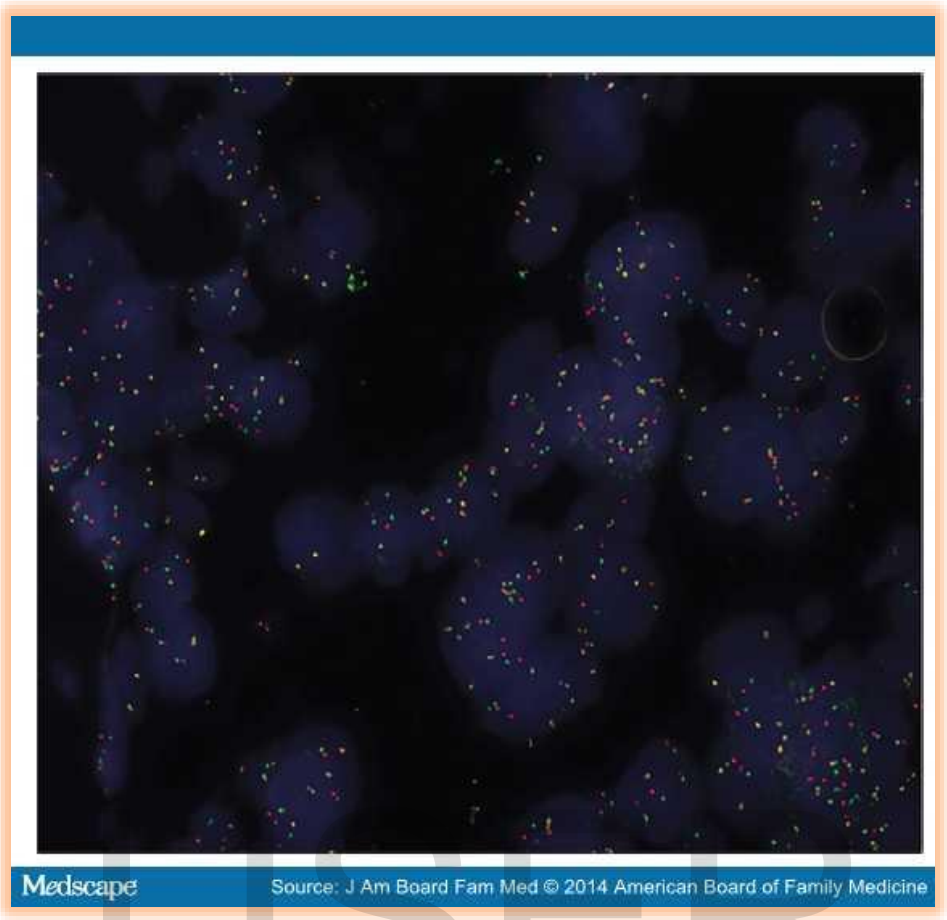

Figure 9. positive lung cancer by using fluoresecent in situ hypridization(FISH) .(Rolfo et al.,2015)

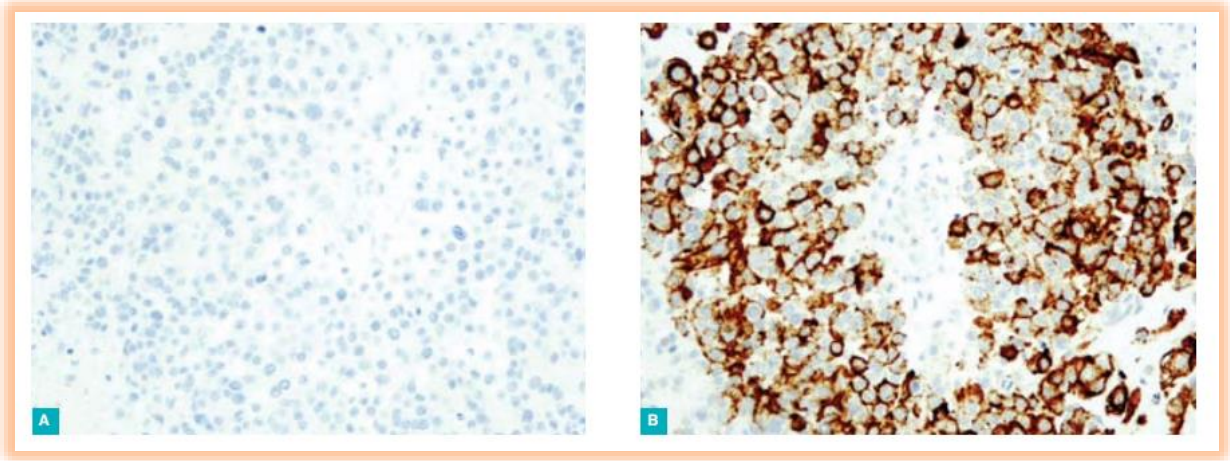

Figure 10. A, small signalls to HER2 by using IHC and B, multisignalling by CSA in immunostaining. (Tani, Hatanaka and Hashizume.,2006).

CONCLUSION:

In the near future, the fast exploration of targeted therapies will trigger to significant changes in personalized treatment for cancer. It is highly approved than histopathology contributes with the vast majority of diagnosis cases either by IHC or FISH but the pathway towards develop a new techniques which capable to give an ideal detections in short time, less cost, less expertise such as microarray or another tests which still under development. For example, multigene assay or as it known multiparameter gene expression which ideally detect on the gene expression in tumour specimens, such as in case of identify estrogen, progesterone receptors positive breast cancer will help the patient to select between using chemotherapy or hormone therapy (NCI,2013). These tumours markers tests will develop in the future considerably for high level of detection, identifying the response and also treatment targets. As we can figure out in this graph below the prediction facts for several types of cancer in case of lack of treatment or diagnosis.

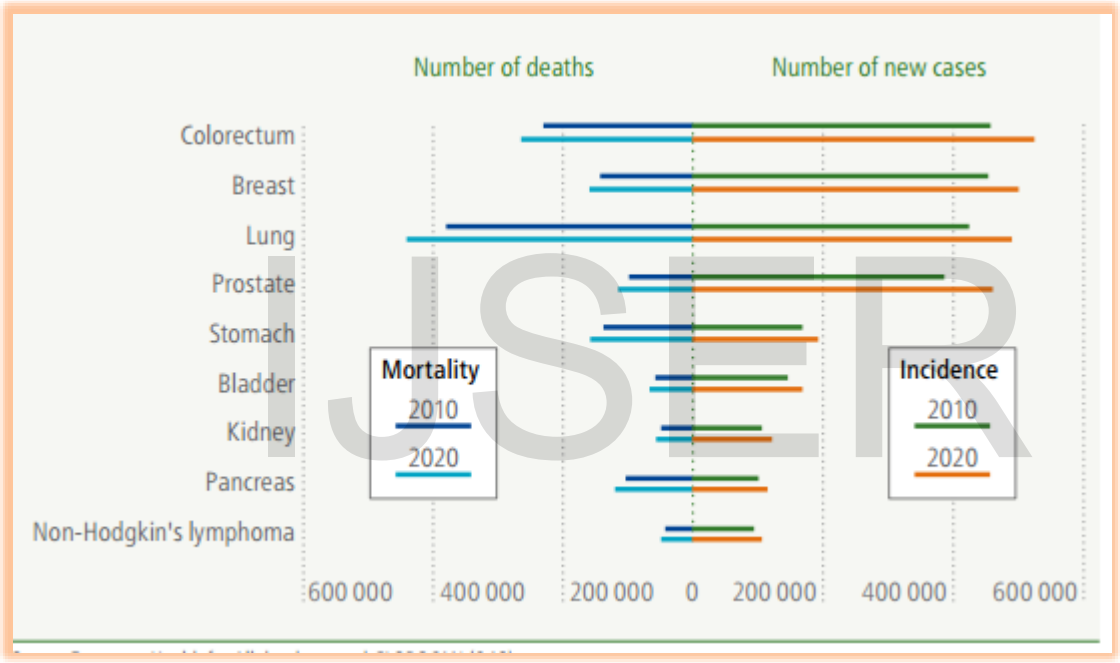

Figure11. the expectations rates of deaths and new cases during 2010 to 2020(The European health report ,2012)

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